Y. N. Pavlovsky

HUMAN DISEASES WITH NATURAL FOCI

FOREIGN LANGUAGES PUBLISHING HOUSE
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EDITED BY ACADEMICIAN Y N PAVLOVSKY

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This book contains an outline of the basic concepts of the theory of human diseases with natural foci, with a detailed summary of contemporary data on tick borne encephalitis, Siberian tick typhus, tick borne relapsing fever, *leptospiroses, tularemia, and leishmaniases* Field and laboratory research techniques, as well as methods of prevention and control, are described

*The book is intended as a manual for epi*demiologists, microbiologists, virologists, parasitologists, physicians employed at anti-epidemic stations, medical arachno-entomologists, and *teachers at medical institutes*

TRANSLATED FROM THE RUSSIAN BY D ROTTENBERG

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Introduction

So far, the concept of natural focality has been developed in its relation to infections transmitted from animals to man and parasitic diseases, helminthiases in particular. The field it covers is extremely wide, involving a considerable number of diseases, many of them not fully explored, and enormous territories that contain diffuse natural foci which are either intact or, as in most cases, affected to a varying degree, directly or indirectly, by the activities of man. The latter tend either to weaken and suppress or, inversely, to activate such foci, sometimes even originating new foci of anthropurgic origin.

Accordingly, practical work on diseases with natural foci requires a knowledge of the following:

(1) focal structure, location and environment;

(2) social factors determining human exposure in focal areas;

(3) combined specific and non-specific measures to be adopted for prevention of the disease.

The respective therapy, although equally vital, cannot be examined hereunder, as constituting a matter of purely clinical interest.

The substantial experience accumulated on the subject was utilised in 1939 to formulate the theory of diseases with natural foci.

The present book contains informative and methodological data for work on the epidemiology and epizootology of a number of diseases with different types of foci either confined to their natural environments, or penetrating more or less easily into the direct neighbourhood of man, sometimes even into human dwellings. The solution of the tasks put before the Soviet public health service by the current Seven-Year Plan, will necessitate extensive and diversified research on the subject throughout the territory of the U.S.S.R. The problem being as multifold as it is, the work will require the participation of different specialists. Success will follow on the correct positioning of scientific forces and proper coordination of efforts.

The results achieved to date are due, primarily, to the sound faunistic and ecologo-parasitological grounding of the theory, which provides a firm basis for special virological, microbiological and epidemiological studies and preventive measures which, taken as a whole, must solve the problem as it stands today. However, as always in science, the solution of the problem in the first approach puts forward a range of new problems, more concrete, and, perhaps, more difficult to solve.

The compilers of the book were confronted from the outset with the fundamental difficulty of determining the structure of its contents. The question was: (a) whether or not it should be a complete manual on all aspects of the problem, and (b) which class of specialists should it serve —the field or laboratory worker with considerable experience in the subject, or biologists and physicians taking their first steps? The former, most probably, would look for concrete instructions of special character, while the latter would expect information, though essential to themselves, yet presenting no novelty to workers with greater experience in the same fields. The difficulty of satisfying both categories is self-evident, since the book can in no way lay claim to encyclopaedic scope.

As a first attempt at compiling an informative and methodological guide, the book's purpose is to acquaint the reader with the essence of the problem as a whole, to point out its related components, and give an idea of the general and specific lines along which investigations should be conducted, in such a way that each specialist might ascertain his place in the broader complex of work, and visualise the relationship of different specialities in elaborating the general and specific aspects of the problem

Academician *Y N Pavlovsky*

THE CURRENT STATUS OF THE THEORY OF NATURAL FOCALITY OF HUMAN DISEASES

I

At present, the principles of the theory of natural focality apply to the following diseases

(1) *viral* seasonal encephalitides—tick-borne (including Scotch louping ill) and mosquito borne (Japanese), two-wave meningoencephalitis, lymphocytic choriomeningitis, pappataci fever (probably), rabies, psyttacosis and ornithoses

(2) *rickettsioses* Rocky Mountain spotted fever (U S A), Japanese river fever, South East Asian scrub typhus, Mediterranean fever, rickettsioses of Asia (Siberia and Far East),

(3) *spirochetoses* tick-borne relapsing fevers of Africa and the countries of the tropical zone, Persian tick-borne typhus tick borne spirochetoses of Central Asia, Transcaucasia and Northern Caucasus,

(4) *leptospiroses* with different forms of causative agents,

(5) *mycoses* actinomycosis,

(6) *bacterioses* plague, tularemia, brucellosis, anthrax, listerellosis

(7) *protozooses* cutaneous leishmaniasis (desert form), kala-azar, African sleeping sickness, Chagas' disease of South America, toxoplasmosis,

(8) *helminthiases* trichinellosis, diphyllobothriasis, sparganosis, echinococcosis, opisthorchosis, clonorchiosis, paragonimiasis, schistosomiasis, etc ,

(9) *arachnoses* animal mange (transmissible to man)

(10) *entomoses* myiases (Wohlfahrt's fly) dermatobiasis (South America)

Most of the above have been revealed in the U S S R , although, doubtlessly, there are more to be discovered

Hence, the immediate objectives of medicobiological research are (a) exploration for as yet unrevealed diseases with natural foci (b) detailed investigation of the structure and environments of foci already revealed, (c) determination of their geographic and topographic distribution (d) experimental and field research on methods of control and possibly, elimination, (e) elaboration of individual and mass scale preventive measures for workers and visitors in areas known or suspected to contain natural infective foci

Hereunder, we shall examine the procedural systems which are common for all research on natural foci of infection

II

In its first concrete formulation, the theory of natural focality, illustrated with examples of the fundamental types of corresponding diseases, was expounded by the author of this paper at the general meeting of the U S S R Academy of Sciences on May 29 1939, and was published in *Vestnik Akademii Nauk S S S R* (Annals of the U S S R Academy of Sciences), No 10 for the same year

In the 20 years which have elapsed since then, the theory has been widely acclaimed, and its basic principles applied in epidemiologic research by various institutes in many parts of the Soviet Union In Leningrad such work was done by the general biology and parasitology department of the Kirov Military Medical Academy, recently awarded the

Order of Lenin In due course, the departments of micro biology, epidemiology, infectious and nervous diseases of the same Academy also took part in the work Faunological, ecological and methodological work of lasting importance was carried out by the Zoological Institute, U S S R Academy of Sciences, with the participation of entomologists, parasitologists and ornithologists On the establishment of the former Gorky All Union Institute of Experimental Medicine (VIEM), the author was invited to organise its parasitology department, whose sphere of activities was shortly afterwards extended to include the organisation of parasitological expeditions On the transfer of the aforesaid Institute to Moscow, the department of parasitology had to be set up anew, considerably ex panding the scope of expeditional research

The institution of a number of affiliated research stations and branches of the Academy of Sciences of the U S S R, permitted the extension of such research to large areas in Central Asia (particularly Tajikistan, Turkmenia, etc) With the endorsement of the U S S R Academy of Medical Sciences, the aforesaid department was transferred to the Gamaleya Epidemiology and Microbiology Institute under the auspices of the said Academy, and then reformed into the department of diseases with natural foci

Extensive work on the subject is being done at the Zoology and Parasitology Institute of the Kazakh S S R Academy of Sciences, the Saratov "Microbe" and many other institutes in different parts of the U S S R

It is heartening to witness the increasing influence of the theory on the work of scientists in other socialist countries, particularly Czechoslovakia (and through her aid Yugoslavia) and Poland The first successful investigations have been accomplished in the Chinese People's Republic

The theory of diseases with natural foci has been included in parasitological and epidemiological textbooks and manuals With the impressive growth in literature and numerical increase of medical and biological institutions directly or indirectly engaged in developing various associated problems, new branches of research appear, while the older assume more definite contours, contributing to overall theoretical and practical progress in the field

To offer an idea of the most feasible lines of future research, it will be of benefit to re-outline the essentials of the theory, defining, in the first place, the modern concept of a natural focus of disease

A natural focus is a section of terrain marked by a definite biogeocenosis which is characterised by more or less clearly defined biotopes, and the presence of biocenoses which, apart from indifferent components, include animals acting as hosts for the causative agent and as donors for blood sucking *Acarina* or other insects that may become vectors and transmit the pathogen to susceptible animals (recipients) The pathogen is transmitted alimentarily, as the vector sucks first the blood of the donor and then recipient. The intervals between the two blood meals may vary widely, depending on the parasite's biology (metamorphosis, frequency, or inversely, extreme rarity of blood meals) and on abiotic environmental factors promoting or at least not hindering the pathogen's development in the vector organism, nor inhibiting the latter's need for fresh blood The focal biocenosis proper and the relationships within it were originated in the course of evolution of live beings on the given geosubstratum independent of the evolution of man, sometimes, probably, even prior to his appearance on earth

The natural focus of a given disease may exist only while the vector continues to transmit the pathogen from donors to recipients In this manner, the circulation of the pathogen is maintained, and if it stops, the focus ceases to exist

A natural focus may become a source of infection for man when non immune individuals enter the focal area with hungry pathogen carrying vectors present. These, being attracted by such welcome prey as man, attack, draw blood and simultaneously deposit the pathogen contained in their saliva in his body This happens in the course of the pathogen's circulation in the vector's body, when the portal of exit are its salivary glands and perforative mouth organs As a result of attack by an infected vector, the human victim may fall ill, provided that the pathogenic dose introduced is sufficient and the given pathogen strain is not weakened due to its own variability or other causes In its clinical form, the resultant disease may range from *formes frustes* to severe and sometimes lethal cases

Despite the introduction of the pathogen into the body, the disease may not be clinically manifest Thus, when inoculating white mice with a suspension of the virus of tick borne encephalitis prepared from the crushed viscera of an infected *Ixodes persulcatus*, most mice contract the disease and die with the typical symptoms, whereas some are completely unaffected, remaining so at all further lethal inoculations, i e , obviously becoming immune Hence, the virus of tick-borne encephalitis may have a dual effect, either killing or immunising the victim (Y N Pavlovsky and V D Solovyov)

The aforesaid suggests a conclusion of major importance for correct assessment of the results of experimental infections with apparently negative results, viz , such experiments may not be set down as failures without the most careful analysis After inoculation, animals not contracting the disease should be thoroughly examined, which may be done in different ways So, when infecting guinea pigs with blood containing the pathogen of tick borne relapsing fever, part of the animals may reveal no spirochetes even in repeated thick film blood tests But if, subsequently, a cerebral suspension from the same animals is injected into healthy specimens, the latter may sometimes prove spirochete carriers Hence it follows that the first experiments must have occasioned a kind of latent infection which became manifest in the second series These findings necessitate for caution in determining, for example, the percentage of spontaneously infected ixodid vectors in the tick population under survey

Experiments showing the immunising function of visceral suspensions from ticks infected with tick borne encephalitis, provided the clue to a hitherto mysterious phenomenon observed by epidemiologists The serum of people never afflicted with spring summer encephalitis, revealed anti bodies to the latter's infective agent, which was testified by neutralisation of the active virus by a definite titre of serum The real nature of this important phenomenon remained obscure until the described experiments on white mice provided an explanation Apparently, the persons in question had visited localities where they had fallen prey to spontaneously infected vector ticks which had served in their case, as a syringe with live vaccine

Experiments with the yellow fever virus employing vector mosquitoes sustained under various temperatures, conclusively showed that, in colder environments, incubation time increases until a certain limit when the vector no longer infects experimental animals, but renders them immune to the disease Similar changes in the effects of the pathogen of tick borne encephalitis are evidently unrelated to temperature, since within certain bounds this virus is indifferent to cold, at any rate, in nature The early spring outbreaks of the disease in man are due to attacks of hungry spontaneously infected ticks emerging from hibernation The amount of ticks participating in the attack and hence, the dose of virus introduced, may likewise be important, but there might also be other factors as yet hypothetical

The variability of the virus proper may be important as well Of course, this question belongs, mainly, to the sphere of virology, but, justifiably, certain ecological considerations may also be put forward Practically, the virus of tick borne encephalitis is always contained in the body of a mammal or bird host or ixodid vector In the course of the pathogen's circulation in the focal biocenosis, each of these *animals in turn serves as its environment* And, since the tick vector of this disease may feed on many species of mammals and birds, in practice, the viral strains circulate through a wide range of animals, forming such chains as Amur hedgehog tick chipmunk tick-wood hen tick wolf cub, etc Biochemically, the aforesaid are non-equivalent, which naturally prompts one to ask, might not such a varied succession of hosts affect the biology of the given strain? In other words the genealogy of different virus strains may vary widely in regard to transition from host to host

Do the specific features of different virus strains remain immutable in the course of circulation through the bodies of various species of mammals, birds and reptiles? One of the primary tasks of experts is to determine the importance of these animals as recipients of the virus borne by infected *Ixodes* At every given moment, the genealogy of a virus is a matter of the past, its sequence being impossible to reconstruct Nevertheless, a certain approximation to an assay of the effects of a host body as an environment on the qualitative status of the virus may be achieved in laboratories with a sufficient choice of experimental wild animals The department of diseases with natural foci of the Gamaleya Epidemiology and Microbiology Institute, U S S R Academy of Medical Sciences (medical zoology and parasitology dept , former VIEM) *possesses a vivarium with wild animals and birds, where* experimental investigations have been made on a number of aspects of the epidemiology of transmissible diseases and the biology of animals serving as reservoirs for various infective agents Considerable organisational achievements have been gained in the Zoology and Parasitology Institute of the Kazakh Academy of Sciences, Alma Ata, where a field station has been set up with a vivarium of wild animals hitherto unutilised in laboratories and maintained under conditions almost indistinguishable from their natural environments Here experiments may be staged on much larger wild animals than ever employed for such purposes before.

Dealing with the problem of the possible variability of the pathogens of diseases with natural foci (viruses, etc), it is necessary to consider yet another aspect of the circumstances under which the pathogen exists in a vertebrate host. If, in experiments, the birth of an animal takes place under strictly aseptic conditions, the newly born animal may be sterile and continue as such if afforded aseptic maintenance, food and water When released into customary conditions, the animal soon becomes populated with bacteria, fungi, Protozoa, and later, helminths All such population, which is alien to the animal and inhabits its open organic systems, is known as a parasitocenosis

The terms parasitocenosis and biocenosis are by no means synonymous The latter, as a community of various flora and fauna, is the result of evolution and may exist more or less continually without appreciable change. On the other hand, a parasitocenosis forms under conditions arising during the individual development and growth of the host, and is specifically unstable and varies rapidly The microbes pathogenic to the given organism, on entering the latter, are surrounded by the species comprising the parasitocenosis of the respective organic system, which may lead to different consequences Some components of the parasitocenosis may be indifferent to the infective agent, others may inhibit its pathogenic activity (live antibiotics), while still others may prove to be synergists, enhancing its virulence Such a multiformity of intraspecific relationships in the parasitocenosis may either cause diseases of different severity in the macroorganism, or make it a symptomless parasite carrier In cases like these we observe variations in the infective agent's virulence occasioned by other species of the same parasitocenosis systematically differing from the former At present, convincing data are available showing the positive and negative relationships between bacteria and Protozoa on the one hand, and Protozoa and parasites on the other, in the intestine of a child

The above shows that in the course of diagnostic or experimental studies, analysis should not be restricted to disclosing the conventionally recognised causative agent, but should strive likewise to identify other components of the parasitocenosis All this is important not only for an understanding of the pathogenesis of the given case or the course of the given experiment, but also for the success of therapy, possibly requiring additional measures such as expulsion of helminths which may handicap the course of otherwise successful treatment in various diseases caused by viruses, bacteria and other infective agents The aforesaid considerations prompted the author to a synthesis of the concept of diseases with natural foci and the theory of parasitocenoses (Y N Pavlovsky)

III

In the numerous expeditions conducted during the last twenty years by various medical institutions under the auspices of the U S S R Ministry of Public Health (former VIEM, etc) which involved multilateral investigation of problems as yet undealt with by the public health service, e g , the "cerebral diseases" of the Far East, which proved to be seasonal transmissible encephalitides, as well as other maladies, the active participation of zoologists, ecologists and parasitologists proved highly beneficial The latter's participation was necessitated by the fact that the cardinal epidemiologic characteristics of these diseases proved essentially biological, their foci being harboured in nature and occurring in such "wild" places where man had never set foot

From the very outset, the studies revealed cases of certain specific diseases encountered in unpopulated areas of the taiga, desert or steppe This led to the *a priori* conclusion that man could receive the infective agents from no-where else but sources of infection concealed in nature Another cause for meditation was the more or less clear cut seasonal character of the outbreaks, the fact that they began and developed in spring and summer All these findings and considerations led to the elucidation of the endemic mechanism of the whole group of diseases

The entire course and results of these studies demonstrated the benefit of the participation of zoologists and parasitologists in the solution of cardinal problems of the epidemiology and epizootology of diseases which initially exist as zoonoses, but later, under definite conditions of place and time, may become zooanthroponoses A considerable part of this work fell to the lot of such institutions under the author s supervision as the general biology and parasitology department of the Military Medical Academy, the parasitology department of the Zoological Institute (and later the institute itself) of the U S S R Academy of Sciences, the parasitology department of the former VIEM and the medical zoology and parasitology department (now department of diseases with natural foci) of the Gamaleya Epidemiology and Microbiology Institute of the U S S R Academy of Medical Sciences, and certain other institutions However, the benignity of such participation could not have emerged immediately, without prolonged preliminary research and more or less definite orientation, which served, in aggregate, as the methodological premises for the ultimate successful solution, in collaboration with medical experts of an epidemiological problem of major importance The said preliminary investigations had pursued their own purposes and were initiated for different reasons but subsequently proved not only useful, but virtually indispensable in coping with new, profoundly important tasks We speak of these investigations on the basis of concrete personal experience, and with the sole intention of demonstrating the need for the competent participation (on equal terms) of expert biologists in the all round elaboration of major medical problems

The seasonal nature of diseases occurring in unpopulated areas, led to the supposition that such diseases were transmitted from animals to man, and hence, spread through some kind of vectors—blood-sucking ticks or insects

Consequently, it is essential to be able to precisely define the species of the surmised vectors, which is made possible by the use of properly compiled identification reference reflecting more or less exhaustively the composition of the tick and insect fauna The task of compiling such reference lies with zoologists specialised in systematics and faunistics. Identification should be carried out with all possible care Whenever doubts arise, the investigator should consult competent specialists, laying aside the tick and insect specimens in question for the latter's examination Unfortunately, the general zoological knowledge of many physicians is extremely limited, owing to the replacement of medical zoology departments in medical colleges by departments of general biology

All medical workers jointly engaged in various activities, especially field or experimental research, should invariably be governed by the following dictum The specific identification of any animal which the physician has to do with in the course of diagnostic, hygienic and epidemiological investigations, should be carried out with no less fundamental accuracy than the determination of bacteria and other microorganisms In responsible or newly initiated investigations such work should be done in consultation with expert zoologists, conducting it with the combined participation of mammalogists, ornithologists, parasitologists, etc

It is not enough, however, to be able to determine the species of this or that parasite, vector or host It is necessary, likewise, to know where to find it in the environment—whether in the immediate surroundings of man, or in nature Such searches are conducted to establish the potential epidemiological hazards of a given area for medical and practical purposes Work of this kind requires familiarity with the techniques of collecting ticks, insects, rodents and other animals, acquaintance with methods of live maintenance for observation of the life cycle and biology, for various experiments (testing insecticides, repellents, etc.) and for parasitologists and epidemiologists to determine the spontaneous or experimental incidence of this or that infective agent. Hence, it is incumbent upon zoologists of different specialities to draw up special instructions, manuals, methodologic handbooks, etc., for the above-named purposes. Fortunately, such literature is readily available.

Territorial surveys, undertaken as part of more comprehensive investigations, may necessitate not only the collection of animals, but also the establishment of their habitats, analysis of the abiotic environmental factors and specific composition of the respective biocenosis, and elucidation of the animals' interbiocenotic relationships. All these problems are the subject of descriptive and experimental ecology and biocenology. The findings of such investigations provide the microbiologist, virologist and parasitologist with excellent material for tracing the circulation of this or that infective agent among the members of the biocenosis and determining the key participants in the process. Such analysis follows as the termination of combined investigations in a natural focus of disease, revealing its epizootological structure and the conditions under which the zoonosis represented in the focus may develop into a zooanthroponosis.

An essential part in preparing for such combined investigations was played by our own work conducted in 1923-1925 in the former Novgorod district with the tick *Ixodes ricinus* (Y. N. Pavlovsky, D. I. Blagoveshchensky, B. I. Pomerantsev, N. I. Alfeyev, et al.). Its purpose was to determine the animals on which the parasite fed and establish the mosaic of the tick's distribution over a selected part of the area under survey, which depends on the features of its habitats—the local vegetation and animal population. An "infestment calendar" was drawn up for the tick's principal hosts among the wild fauna, as well as special tables showing the dynamics of cattle infestment in their relation to grazing and to the way the cattle were utilised by man (effects of the social factor) Special tick nurseries were maintained through the winter to establish the length of tick metamorphosis, which proved considerably different from laboratory findings All these measures were undertaken for the study of *Ixodes ricinus*, the specific vector of cattle piroplasmosis (babesiasis) and the experimental development of control measures as part of preventive campaigns against outbreaks of the disease among cattle

The works on ticks and other ectoparasites, later continued by the All-Union Plant Protection Institute of the Lenin All Union Agricultural Academy, are described in a compendium entitled *Cattle Pests* (U S S R Academy of Sciences, 1935) A notable item in the latter is the classic work of B I Pomerantsev "On the Origin of Tick Foci in the Leningrad Region" whose significance becomes all the greater with the establishment of the role of *I ricinus* in transmitting the agent of tick borne encephalitis The research techniques worked out in the former Novgorod district for investigations on *Ixodes ricinus*, and the respective methods for the study of the genus *Dermacentor*, were fully applied in Far Eastern expeditions (1937-1941) devoted to seasonal encephalitis Apart from other reasons, this was thanks to the assistance of G Serdyukova, a partaker in the Novgorod investigations on ticks, who participated in expeditions on encephalitis carried out in the Khabarovsk district and in the Suputinsk forest reserve further south

Of all our numerous expeditions, the highest standards, as regards coordination of zoological and parasitological research and rational employment of expert personnel, were achieved in the three year studies made in the Gissar valley near Dushanbeh (Tajikistan), organised to discover the tick vector of bovine haemosporidioses and to develop specific prevention techniques The expedition was sponsored by the Zoology Institute of the U S S R Academy of Sciences and the Tajik base (later branch) of the same in Dushanbeh

The principal work was carried out by zoologists, among them the ornithologist A I Ivanov, the herpetologist S A Chernov, the parasitologist B V. Lototsky and the veterinarian I G Galuzo, Dr Sc (Biology)

The zoologists explored the area of the valley and the bordering flanks of the Gissar mountain range The territory was divided into zones according to morphology, flora and cultivation The zoologists shot and trapped specimens of vertebrate fauna, noting the sites of collection The result of these works was a complete record of all the vertebrate fauna of the valley, drawn up by zones Each of the animals was identified by expert zoologists, catalogued with reference to the site of collection, and delivered under identification numbers to the parasitological laboratory Here they were carefully examined by the laboratory staff, who removed all ectoparasites, chiefly ticks, and took blood tests, after which the carcasses were returned to the zoologists All the received information was entered in the journal together with the amount, species and metacyclic stages of the removed ectoparasites The materials obtained were conserved for museum display or employed for experiments The entire work proceeded with conveyor-like regularity, and in the shortest possible time provided such a wealth of material as no singly working parasitologist could ever hope to collect A distinctive feature of these studies was our endeavour to conduct the work throughout the year, simultaneously observing domestic animals maintained under like conditions

A complete parasitologic novelty were regular winter inspections of domestic animals for ticks with special note of the places where cattle were maintained in the winter, which in those extreme south easterly parts of the U S S R is comparatively mild

As regards organisational standards the entire system of research in this multipurpose, but basically epizootological expedition, as yet remains unsurpassed In the course of the work, we were able to determine the valley's ixodid fauna (altogether 16 species), as well as the stage to stage calendar of their life cycle, the metamorphosis of individual tick species and their seasonal association with various species of mammals, birds and domestic animals The expedition also located the abodes of replete ticks detached from their hosts, establishing the respective habitats and microhabitats where moulting, egg laying and hatching took place We likewise made electrometric assays of temperature and humidity variations for different seasons and even, in certain cases, different hours of the day and night A special summary table showed the habitat, time, quantity and host of the larvae, nymphs or females of each tick species parasitising the particular host The vector species of haemosporidioses (Taylor's disease and piroplasmosis) being known earlier and the vector ticks' preying time being reflected in the summary tables, an idea could be formed of the periods of cattle infection and the pasturing sites where cattle were liable to contract the disease from parasite ticks

These important data were accompanied by information on the altitudinal distribution of ticks, which is especially vital for alpine countries like Tajikistan In the very first year of investigations, the alpine pastures were found to be free of ticks The transfer of pedigree cattle from the lowlands to these areas saved the animals which had survived the epizootic then devastating the whole valley

The extension of ecologofaunistic research on the altitudinal and regional prevalence of *Ixodidae* over the entire territory of the republic, enabled the latter to be classified into regions actually and potentially hazardous or non hazardous in regard to epizootics A special map to the effect was drawn up likewise

On the territory of the U S S R, the various species of *Ixodidae* are the vectors of diseases affecting not only domestic animals, but also man Hence, it is all the more vital to make joint use of epizootological and epidemiological information in concretising and modifying the results of investigations on pests which are of common interest to cattle husbandry and public health, especially with a view to adopting coordinate measures for control and prevention of human and cattle diseases prevalent on one and the same territory Unfortunately, as yet, the necessity of such cooperation has not been fully realised

The use of the aforesaid technique of faunistic and ecologoparasitological field work combined with laboratory research, resulted in the discovery of natural foci of the desert form of cutaneous leishmaniasis (Pendinski ulcer, Borovsky's disease, or, in Turkmenian, *murgabu*) and the successful accomplishment of an experiment in eliminating such a focus by poisoning and blocking multitudinous gerbil burrows—the elementary foci of this disease (N I Latyshev) An important preliminary for experiments of this kind was the discovery by P Vlasov and P Petrishcheva of the existence of *Phlebotomus* in nature—in burrows and other harbourages outside domestic and service premises In regard to the natural foci of tick borne relapsing fever in Central Asia, it was demonstrated that the principal members of such foci may migrate to crude cattle shelters and human dwellings and there form new foci

The extensive works on tularemia, conducted in the U S S R revealed the existence of natural foci of this disease associated with ixodid ticks inhabiting on pastures For all the extreme multiplicity of routes through which tularemia may be contracted, the principal part in maintaining its causative agent in nature is played by *Ixodidae* (N G Olsufyev)

The result of our year round observations on *Ixodidae* in Tajikistan was a discovery of major practical importance The tick vectors of the pathogens of cattle diseases hiber nated on cattle, which apparently served them as shelter during the comparatively mild local winter Another circumstance of economic interest, was that cattle spent winter not on grazing grounds but near farms, where the ticks wintering on cattle concentrated as well This offered an opportunity to eliminate the ticks together with all actual vectors among them, employing various dry acaricides (dust, etc)[1]

As a final practical outcome of all these and associated investigations, special instructions were drawn up on the mandatory control measures against diseases transmitted by *Ixodidae* on the territory of the Tajik S S R[2] The organisational principles of research and the rational cooperation of zoologists—specialists in faunistics, ecology, para sitology and epizootology, offer an exemplary model, fully applicable methodologically to epidemiological expeditions and easily modified with regard to the area, time and concrete purposes of the new work in hand

It is essential to make use of the extensive new literature on the subject, e g , individual volumes of *The Fauna of the U S S R* , and the specially published series of methodological aids (a) *Handbook for Forest Belt Workers*, (of which directly concerned with parasitological and other research on diseases with natural foci are issues Nos 1, 3, 6, 10, 13, 14, 15, 16, 17, 21, 22), (b) *Field and Laboratory Worker's Manual* (several issues) Of the non-serial publications of the Zoology Institute, see the *Manual on the Collection and Study of Fleas* (1927), *Manual on the Collection and Study of Ixodoidea*" (1928), *Manual on the Collection, Study and Maintenance of Mosquitoes* (1925, 1927, author Y. N. Pavlovsky). The first two books have also appeared in the series: *Handbuch der biologischen Arbeitsmethoden* (Abderhalden) (Abt. 9, Teil), Heft I, 11-96, and, in the same series, Seite 97-160, 1931.

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[1] Y Pavlovsky and B Pomerantseva 'On the Transfer of Cattle from Summer to Winter Pastures on the Western Flanks of the Alagez' From *The Transcaucasian Parasitological Expedition in Armenia* Proc Conf on Pedigree Cattle Protection (CPCP) Trans caucasian series Issue 11, U S S R Academy of Sciences 1934

[2] Y Pavlovsky and B Lototsky *The Control of Haemoparasitic Diseases of Agricultural Animals in Tajikistan* Tajikistan State Publishing House Dushanbeh 1951

In the U.S.S.R. Academy of Medical Sciences the author organised the issue of a series of methodological handbooks entitled *Aids to Medical Workers Working on the Great Projects of Communism*—nine of which were published in 1952 and one in 1953. Besides that, separate publications of methodological nature were issued by the State Medical Publishing House. The U.S.S.R. Ministry of Public Health has printed methodological aids and instructions on various diseases. A number of periodicals (journals, transactions of various institutes) likewise contain material on diseases with natural foci.

A section on diseases with natural foci has been organised in *Abstracts of the U.S.S.R. Academy of Sciences* (geographical series). Much useful material may be derived from the *Communications of Parasitological Expeditions Sponsored by Institutions of the U.S.S.R. Academy of Sciences, collections of papers published by Republican Academies* (Kazakh, Tajik, etc.). Abstracts and proceedings of conferences on parasitological problems held by the U.S.S.R. Academy of Sciences, the Gamaleya Epidemiology and Microbiology Institute of the Academy of Medical Sciences (formerly VIEM), etc.

Direct participation, both organisational and practical, in the work of expeditions on seasonal encephalitides[1] permitted us to make extensive use of our experience in organising research on a faunistic and ecologo-parasitological basis, which has been of much benefit in elucidating the epidemiological features of tick-borne encephalitis and developing non-specific prophylactic measures against the disease. The same refers to the work on tick-borne typhus fevers in Siberia, carried out by expeditions under the sponsorship of the parasitology department, VIEM (later the Gamaleya Institute of Epidemiology and Parasitology, U.S.S.R. Academy of Medical Sciences), which demonstrated the natural focality of the said diseases (S. P. Petrova-Piontkovskaya, O. S. Korshunova, S. M. Kulagin, N. I. Alfeyev, G. V. Serdyukova).

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[1] In 1938 the author was placed at the head of an expedition on tick-borne encephalitis, and in 1939 headed the team responsible for studying the factors conducive to the existence of natural foci of the said disease in an expedition led by I I. Rogosin. The first expedition on the subject, organised in 1937 and headed by L. A. Zilber, included a team of entomologists and parasitologists manned by the general biology and parasitology department and department of microb ology, S M Kirov Military Medical Academy (field work supervisor A V. Gutsevich)

The benefit of employing coordinated faunistic, ecological and parasitological studies as a basis for subsequent multilateral investigation of major epidemiological and epizootological problems, may be illustrated by other examples.

A subject of special note are the foci of certain diseases which appear in nature as a result of human activity, and hence, in contrast to natural foci, may be defined as anthropurgic, i.e., to a certain extent, originated by the activity of man. For instance, in a number of cases, wild animals such as rodents and even snakes were found to be spontaneously infected with a causative agent of brucellosis (Samsonov). Further, it was established that various species of ixodid ticks, after feeding on animals whose peripheral blood contains *Brucellae*, may become not only hosts, but vectors of the agent. The latter happens when they suck the blood of healthy animals, which in their own turn may serve as pathogen donors for fresh ticks (I. G. Galuzo, M. M. Rementsova).

Individual observations in nature, together with the results of numerous and varied experiments, allowed us to construe the following conjectural sequence of events. A given grazing ground is inhabited by *Ixodidae* and small animals such as rodents, etc. On this pasture, a cattle herd including animals with *Brucellac* in the blood, are put to graze The hungry ticks occurring at the time on the pasture, attack the diseased cattle and, together with the latter's blood, receive the infective agent At their next trans stage blood meal, the pathogen carrying ticks infect susceptible specimens among both the natural inhabitants of the grazing ground and the healthy agricultural animals brought to graze Henceforth, the pasture becomes a natural focus of brucellosis as a result of human activity expressed *in the successive pasturage of diseased and healthy* animals The healthy herd is put to graze after the diseased pending a certain interval during which the pathogen is maintained in the bodies of vector ticks Thus, a brucellosis focus appears due to the joint effect of natural circumstances (ticks and wild animals inhabiting the pasture) and the activity of man, the latter being responsible for the arrival of sick animals and the subsequent pasturage of healthy ones Intermediately, we have the infection of ticks with the pathogen and the eventual possibility of their transmitting the pathogen to healthy animals

Thus, quite conceivably, a natural, or rather, anthropurgic focus of brucellosis develops The length of its existence presents a special problem, but even its brevity should not cast doubt on the character of its originating causes and the validity of the proposed denomination

What then is the theoretical essence of the process of formation of a new natural focus of disease?

The main requisites for the possibility of its formation are *(a) availability of pastureland not cultivated to the* point of extermination of its natural animal population—some of the species of *Ixodidae* as well as small mammals serving the former especially their larvae and nymphs as sources of sustenance (b) susceptibility of ticks and wild *fauna to Brucella (c) formation of a* biocenosis (including ticks and other pasture inhabitants) of more or less permanent specific composition

All these are the natural factors *prerequisite* for the development of brucellosis foci The very appearance of the natural focus depends on the introduction into the pasture biocenosis of the only missing link—the pathogen of brucellosis contained in the bodies of diseased animals put to graze

The *decisive factor* in the formation of a brucellosis focus on the grazing ground is essentially social viz, the conscious will and activity of man, who brings diseased and then healthy animals to graze on the given pasture

How long such an anthropurgic focus will exist depends on the stability of introduction of the causative agent into the animals composing the pasture biocenosis and on abiotic factors being unopposed to such introduction or to the existence of the biocenosis itself, enriched, as it is, *with the aforesaid new component, i e, the pathogen*

IV

A rational classification of natural foci of disease requires their assessment in regard to factors of biological, abiotic and geographic (topographic) nature, and a statement of the relationship of their origin to the activity of man

1 Natural infective foci may include the pathogen of a single infectious or invasive disease (monoinfectional foci)

2 A natural focus may harbour two or more diseases (di- and polyinfectional foci)

3 The biocenosis of a natural focus of disease may include several species of animal hosts maintaining a single infective agent (polyhostal foci)

4 In the same focus there may be one or several vector species transmitting the same disease (mono and polyvectoral foci, respectively)

5 As regards their age, foci may be ancient or recent

6 As regards distinctions of origin, some natural foci may have developed evolutionally (autochthonous foci), and others as a result of human activity (anthropurgic)

7 When sections of terrain (which we will call land scapes), typical of a given focus, split off on the boundary between two distinct types of terrain (e g, forest and steppe) the resultant insular areas may still contain the respective foci Thus, steppe surrounded portions of a forest proliferating into the steppe may contain natural infectious foci peculiar to forests

Natural foci may exist on a variety of geographic landscapes, or zones The definition of a geographic land scape by L S Berg, states it to be "a combination or association of objects and phenomena where all the features of relief, climate, water, soils, flora and fauna, and, to a certain extent, human activity, unite in a single harmonious entity, repeated typically throughout the given geographic biome "[1]

On the territory of the U S S R, the following zones are distinguished (1) tundra, (2) temperate forest, (3) forest-steppe, (4) steppe, (5) semi desert, (6) desert, (7) alpine

Individual landscapes comprising the zones, are not homogeneous throughout, but contain different habitats associated with separate details of relief and harbouring various biocenoses composed of the flora and fauna common to the entire landscape (zone)

In view of the above, it is logical to associate the natural foci of various diseases with different respective landscapes Thus, natural foci of tick-borne encephalitis are typical of forests of definite botanical composition, located in the temperate biome, the same foci may also occur in forest copses advancing into the steppe (forest-steppe biome) The steppeland areas of the forest-steppe may contain natural foci of tick borne typhus fever or tick-borne typhus, the steppe may also harbour natural foci of the plague, which extend into the desert and semi desert The southern part of the steppeland zone in Kazakhstan the North Caucasus, as well as the semi-desert, desert and alpine areas of the respective latitudes, contain natural foci of tick-borne relapsing fever Burrows occurring in the deserts and semi-deserts of a number of regions in Central Asia sometimes harbour elementary foci of the Pendinski ulcer (cutaneous leishmaniasis) and the plague

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[1] L S Berg *Geographical Zones of the Soviet Union* Moscow, 1947

Such analysis of the relationship between the natural foci of diseases and individual landscapes should be carried out universally and detailised according to the finer topographic division of each given landscape with attention to the special features of localisation of areas liable to harbour biocenotic components typical of a given infective focus

Certain diseases, tularemia in particular, are prevalent in a wide variety of geographical landscapes, which accounts for the former's striking multiformity For further information on the subject, see N G Olsufyev's chapter on tularemia included in this book

On the boundaries between heterogeneous landscapes the respective fauna may happen to be somewhat mixed If such a mixture involves the leading components of biocenoses existing in natural foci of diseases peculiar to both types of landscapes, people occurring in the border area are exposed to the respective infections as well Such instances, however, do not exclude the fundamental connection between the natural foci of various diseases with the landscape typical for each of them The knowledge of this connection is of essential practical importance for the prevention of the respective diseases

V

The enormous territory of the Soviet Union has so far not been sufficiently studied to permit a more detailed division into regions according to features of local pathology. With the reclamation of new areas and utilisation of the resources concealed in unexplored territories and in the bowels of the earth, there is always the possibility of people penetrating into localities with as yet unrevealed natural infectious foci Preliminary surveys of such localities for possible incidence of the foci of zooanthroponoses, are often omitted In these cases, a careful visual appraisal of the characteristics of the local terrain may furnish grounds for suspecting the presence of various kinds of infective foci

The stated facts should incite those concerned to adopt measures for safeguarding personnel against the attacks of blood sucking arthropods liable to include hungry vectors infected with various pathogens Even in case the latter are absent, protection against winged blood sucking vectors will favourably tell on labour efficiency

Non specific protection against diseases with natural foci is all the more important since specific prevention can not as yet be provided, the technology of vaccine manufacture for some of these diseases not being developed So far, there exists no effective vaccine against tick borne encephalitis We have drawn attention elsewhere to the advisability of attempting the preparation of such a vaccine from ticks experimentally infected with the virus, by grinding the latter s viscera in glycerin with the addition of some kind of conservant The obtained substance should be tried out on laboratory animals to determine the vaccine titre It may be hoped that such a vaccine would prove more practicable for mass inoculation of people The aforesaid proposal of the author was published in the *Journal of Microbiology, Epidemiology and Immunology*, and evoked favourable comment in the U S A , it being noted that the vaccine used against Rocky Mountain spotted fever is manufactured on the same principle (Philip) The further development of the problem calls for joint efforts on the part of virologists and parasitologists

As regards non specific prevention of diseases with natural foci, primary significance should be attached to individual protection of people against the bites of blood sucking ticks and flying *Diptera* (punkies) The latter may include infected vectors of viral diseases with natural foci. In the U.S.S.R., investigations on punkies have continued systematically since 1935. There is plentiful scientific material on the identification of component fauna, e.g., in diverse volumes of *The Fauna of the U.S.S.R., Guides for the Identification of the Fauna of the U.S.S.R.*, published by the U.S.S.R. Academy of Sciences, methodological aids on the study of punkie biology.

A large number of insecticides have been put to test. Various ways of their use have been proposed, but an essential fault is that they have not been properly tried out in practice. Only preparations sufficiently tested as to their effects in natural conditions should be turned over for mass manufacture. The authors should bear responsibility for the quality of the proposed chemicals.

In a number of cases, the lack of such responsibility leads to confusion. For example, when Nabokov's smokers became known and the press began publicising their use against punkies, one article contended that the smoke from such appliances supposedly destroys ixodid ticks including specimens infected with the virus of tick-borne encephalitis. This statement met an eager response in localities where prevention of this disease is a matter of urgency. We, personally, have received a handbook printed in the Far East on the preliminary decontamination of future sites of prospecting, research or construction work, which are liable to harbour natural foci of tick-borne encephalitis. It was stated that special teams furnished with Nabokov's smokers should be dispatched to the areas in question, laying the devices in staggered order and setting them on fire. The smoke thus obtained would supposedly kill the ticks, clearing the area for the proposed work. Actually, however, it was established that the mentioned smoke has no fatal effect on ticks, and hence proved useless for the said purposes. Misguided belief in the effects of smoke will doubtlessly divert attention from the simpler, but already well-tried methods of protection against ticks, leaving people in hazardous territories without any means of protection whatsoever.

As evidenced by long practical experience, a highly efficient safeguard against punkies are protective nets (Fig. 1) which are quite simple to make. The material to be employed is a standard fishing net of strong yarn with a mesh of 1×1 or 1.5×1.5 cm, preferably of low grade, as higher grades are uneconomical. The net is cut into pieces 80×60 cm, the margin being hemmed with a ribbon for tying to the hat. The nets are soaked for several hours in a 10 to 15 per cent solution of naphtholysol, with addition of turpentine, then dried in air, rolled up and stored in wrappings or oilcloth bags. When going out of doors the net should be laid on the hat in such a way as to freely cover the head and shoulders, the face being left open. The ribbons are tied round the hat. Owing to evaporation of the repellent, an odorous zone arises between the net and the face, which drives away insects. The repellent effect persists for several days, depending on the weather, the net being subsequently resoaked in the solution and dried.

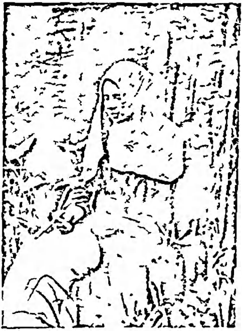

Fig 1 Y Pavlovsky's repellent head-net as used in collection of punkies and mosquitoes on Southern Sakhalin

An improved technique for applying the repellent has been devised by G. S. Pervomaisky and V. K. Nisovkin, who proposed using the substance in the form of dry bars.

Rubbing the bars against the net eliminates the need for impregnation However, this method requires further testing

For protection of the hands, K P Chagin proposed cuffs of the same netting, which are pinned to the sleeves so as to cover half the hands and not hinder work

VI

The existence of a natural focus of disease depends on the occurrence in the biocenosis of organisms whose biocenotic relationships insure the continuous circulation of the infective agent However, a biocenosis cannot develop and exist otherwise than within the given *biogeocenosis*, i e, a definite geographic landscape A biogeocenosis, as defined by Academician V N. Sukachov, ' is formed of a plant community in association with the animal population of a given area of the earth's surface, including the surface proper, with all its special features of atmosphere (microclimate), geological structure, soil and water supply. Taken together, the mentioned components comprise a single interrelated complex "[1]

The cited definition is sufficiently exhaustive, although it may be added that biogeocenoses may also occur in the upper strata of the earth's crust, an example of which are the burrows of wild animals, mostly rodents, which penetrate to a depth of several metres This, however, may in no way affect the general concept of a biogeocenosis, indicating, merely, that in every particular instance the latter should be carefully analysed as to structure, e g, in which there may be a prevalence of vegetable components over animals or vice versa As regards abiotic factors, there may also be noticeable distinctions in seasonal or daily variations from the mean.

Biocenosis may include different numbers of animal species bound by various direct or indirect interrelationships Orientation and purpose of research are of major significance in the study of biocenoses If the aim is to elucidate the epidemiological and epizootological importance of a given biocenosis, the emphasis should be laid on revealing the components acting a distinctive part in the circulation of the pathogen of the disease, whose natural focus should be subjected to multilateral investigations by experts of all the specialities required In areas containing natural infective foci it is necessary to single out the animal donors, vectors and recipients of the causative agent, which represent the leading components of the given biocenosis

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[1] *Bolshaya Sovetskaya Encyclopaedia*, 2nd ed, Vol 5, p 180

A natural focus of disease continues to exist if and while the biocenosis and harbouring area remain intact, and if conditions of microclimate remain favourable throughout the period of continual circulation of the pathogen among the principal components of the biocenosis

A natural focus of disease ceases to exist in the following cases when the key components of the biocenosis fall out and the circulation of the causative agent stops, when the focal area undergoes unfavourable changes (drainage or irrigation), when its geomorphological harbourage is destroyed (ploughing up and destruction of burrows and other biotopes), when microclimatic factors and especially humidity change drastically (e g , flooding of burrows), when the focus is invaded by *Carnivora* which exterminate the key components of the biocenosis, and when for various reasons the biocenosis itself disintegrates

The viability of a natural focus of disease depends on where and how long the infective agent may survive total or partial disruptions of the biocenotic structure, provided that the latter leave the pathogen's harbourages intact

Ample illustrations in this respect may be cited from the work of numerous Central Asiatic expeditions under the author's supervision devoted to investigation of natural foci of tick borne spirochetosis (relapsing fever) Foci of this disease commonly develop in dwellings and services, crude structures, etc Their maintenance requires the existence of mammal hosts, e g , rats, and tick vectors of the genus *Ornithodorus*

Buildings of local raw brick or blocks are not durable and easily fall to ruin when abandoned or neglected In such cases the rats which they harbour disappear, while the resident *Ornithodorus papillipes*, being averse to migration, remain where they are and starve Observations carried out by A N Skrinnik at the tick laboratory, general biology and parasitology department, Kirov Military Medical Academy, on *O papillipes* from a unique collection of live ticks of the genus *Ornithodorus*, demonstrated that they may starve for 14 years and, if infected with spirochetes of relapsing fever, will transfer the patho gen to susceptible animals at the very first meal

Thus, by comparing laboratory and field observations, it is easy to see why vector ticks, being capable of prolonged starvation, can continually maintain the pathogen of relapsing fever despite the destruction of the respective natural foci When visiting such ruins as described, even by day, a person is exposed to infection from the bites of hungry ticks Hence, the possibility of complete revival of the foci in cases when the aforesaid buildings are used for sheltering cattle, etc

The natural foci of diseases whose key vector are ticks firmly settled in their biotopes and disinclined to migrate to new habitations, exert their effects on man either through direct contact or in case of man's sojourn in the biotope proper where he may contract tick borne spiro chetosis

The natural foci of diseases with actively mobile vectors such as blood sucking flying *Diptera* (sandflies, mosquitoes, etc) may affect people at considerable distances, since the insects leave the burrows serving them for daytime shelter or oviposition and attack human beings Such, for example, is the case with the desert form of Pendinski ulcer (cutaneous leishmaniasis)

During reclamation of deserts and semi-deserts, in the very first summer the sandflies converge from rodent burrows and settle round newly built domiciles, whereby the desert form of cutaneous leishmaniasis is transformed into its equivalent urban form (P A Petrishcheva)

VII

The problem of eliminating infectious and parasitic diseases on the territory of the U S S R has of late been put forward on an increasing number of occasions, not infrequently, as a target for the nearest future The campaign against malaria, however, took dozens of years of hard work on the part of a widespread network of antimalarial institutions—from institutes to local stations and outposts which were staffed by a whole army of physicians—malariologists, medical entomologists, land reclamation experts and auxiliary technical personnel

V V Parin, Academician Secretary of the Presidium of the U S S R Academy of Medical Sciences, in his report "On the Tasks of Medical Science in the Light of the Decisions of the XXI Congress of the C P S U" delivered at the XIII plenary meeting of the Academy (April 1959) said the following "Acknowledging that the problem of eliminating infectious diseases constitutes one of the key targets of the Seven Year Plan, the Presidium of the U S S R Academy of Medical Sciences has decreed the establishment of a committee for promoting the elimination of infections, which has been authorised to draw up a seven-year plan of work directed towards this purpose"

The Presidium has already approved a newly-devised classification of diseases according to the prospects of their elimination The first group comprises *infections in* regard to which there exist the practical requisites for total elimination or suppression of their epidemic forms i.e., for reducing their incidence to the level of sporadic cases (malaria, diphtheria, enteric fever, etc.). The second group involves infections the spread of which is to be cut down drastically, permitting the subsequent implementation of measures envisaging full epidemic control (poliomyelitis, tick-borne encephalitis, ascariasis, tularemia, leprosy, etc.). The third group are diseases, the fundamental requisites for the elimination of which already exist, but which require either additional investigations, or the overcoming of a number of organisational or material handicaps, due to which only a considerable reduction of morbidity and mortality may be planned for the nearest future (acute intestinal infections, whooping cough, epidemic parotitis, tuberculosis, brucellosis, Q fever, etc.). The fourth group covers infections in regard to which the possibility and time limits of elimination cannot as yet be established in view of the existence of still unsolved fundamental theoretical and methodological questions (angina, influenza, infective hepatitis, measles, scarlet fever, chickenpox, German measles).

As regards diseases with natural foci, elimination of the latter necessitates destroying the basic structure of their foci which is responsible for the intrafocal circulation of the pathogen. By and large, the route of circulation of the infective agent is determined by the presence in the focal biocenosis of such key components as animal donors, recipients and vectors of the pathogen. The latter present the biotic factors of the natural focus, their existence and biocenotic relations, however, in turn depending on abiotic environmental factors—climate, microclimate, geomorphological features of the area—i.e., the circumstances underlying the existence of the biogeocenosis harbouring the natural focus of disease. All these, however, are dominated by the activity of man, which directly or indirectly affects the biocenosis of the natural focus and the integrity of the focal terrain. In this respect, the activity of man is regarded as a combination of social factors comprising man's unconscious or purposeful influence on nature

The settlement of previously uncultivated areas, such as the taiga, leads to the disappearance of the associated natural foci of tick-borne encephalitis On the other hand it has been noted that the seasonal collection of berries and mushrooms near settlements is accompanied by fresh outbreaks of the aforesaid disease The latter occurs in places where the natural foci of encephalitis, although considerably weakened owing for example, to the reduced number of vector ticks, have survived

With the increased influx of population into such woodland and prolonged close contact with the vegetation, there is a growing hazard of tick attacks and contagion

The elimination of natural foci of disease presumes the adoption of measures of three kinds affecting (a) the area harbouring the foci, (b) the focal biocenoses and (c) the population proper, viz, (1) educational instruction as to the most likely time, places and circumstances in which people are exposed to the hazard of contracting diseases in their natural foci description of the main features of a natural focus and the precautions to be taken against attacks of vectors of the infective agent, sanitary anti-epidemic propaganda through posters, leaflets popular-scientific literature, films, etc and furnishing every means of individual and collective protection including repellent ointments and nets, automatic spraying devices equipment for treating tick ridden areas with aerosols smoke and insecticides, land reclamation machinery, etc, (2) mass vaccination and provision of material for such

Decontamination of the territory is accomplished in various ways, depending on the character of the associated natural foci of disease The process may comprise a single stage and be effective for one season only, like the burning of grass in steppeland areas in order to destroy hungry *Dermacentor* ticks—vectors of rickettsial tick borne typhus fever which lurk in ambush upon the grass stalks on emergence from hibernation. In some localities such measures have to be repeated every spring, observing the necessary precautions against fire. More radical measures to the same effect, involving, as it were, complete overhaul of the terrain, require the accomplishment of a succession of reclamatory operations such as cultivation of forest areas, suppression of undergrowth, etc., over a number of years. All such measures, which are of direct economic importance, should be carried out with a view to sanitation purposes as well.

The aforesaid recommendations are of a general nature, to be modified in each particular instance in conformity with the basic purpose, place, time and circumstances attending the work.

In conducting operations for control of the natural foci of disease, the experience of earlier undertakings in this field should be borne in mind. Thus, we may call attention to the outcome of a number of expeditions on cutaneous leishmaniasis carried out under the guidance of Professor N. I. Latyshev, sponsored by the medical parasitology department of the former VIEM. After a series of preliminary investigations, an experimental attempt was made at eradicating the natural foci of the desert form of cutaneous leishmaniasis in the neighbourhood of a construction project on the river Murgab (Turkmenia). The rate of infection among workers had been considerable. Daily, about fifty people appealed at the local dispensary for dressing. Sandflies were in abundance and the population slept under bed nets.

The focus was to be destroyed by chloropicrin poisoning of all the gerbil burrows within 1-2 kilometres around the settlement. Altogether, approximately 0.5 million burrows were poisoned and blocked with earth. The result was the destruction of the hosts including gerbils afflicted with cutaneous leishmaniasis, and the extermination of sandflies, vectors of the pathogen, which reproduced and spent the daylight hours in the burrows, the latter, too, being rendered unusable for new gerbils and sandflies The poison was applied in a single operation, the results being checked within a year by a specially appointed commission

The commission stated that the incidence of cutaneous leishmaniasis among adults had been reduced to zero only four boys were ill, and the population slept without bed nets It was revealed however, that new burrows of gerbils had begun to appear around the periphery of the sterilised belt surrounding the construction site, which evidently was the beginning of resettlement of the old area by the pest In the first year, the process had no epidemiological consequences, but it is logical to foretell the resultant ultimate recovery of the old natural focus of Pendinski ulcer

Hence, a single campaign proves insufficient to eliminate a natural focus of a given disease A special service should be set up to keep the focus under control to observe the process of regeneration of the focus on earlier treated territory, and to suppress any initial symptoms of such regeneration This is much easier and cheaper than to reapply the entire control system on natural foci regenerating on earlier treated terrain

The stated considerations are of a general nature their concrete implementation depending on the specific features of every particular disease with natural foci

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TICK-BORNE ENCEPHALITIS

Synonyms Taiga, spring summer, Far Eastern, Siberian encephalitis Eastern tick borne encephalitis, spring endemic encephalitis, spring summer meningo encephalitis, Russian tick borne spring summer encephalitis, Fruhlings encephalitis, encephalite du printemps et de l'été, encephalitis del Sejano Oriente

Tick-borne encephalitis is an acute viral disease with natural foci, mostly affecting the central nervous system, and typically harboured on woodland terrain Its vectors and principal reservoirs are ixodid ticks The disease was first defined as an independent nosological unit by Soviet researchers (L A Zilber, A G Panov, E N Levkovich, A K Shubladze, M P Chumakov, V D Solovyov) in 1937

Incubation lasts for a period of 10 to 12 days, less often varying from 7 to 21 days Occasionally, 1 or 2 days prior to onset, the patient evinces general malaise and slight pains in the muscles of the neck, waist, extremities and head The feeling of weakness in the arms may be accompanied by slight numbness (sometimes in one arm only) A frequent occurrence are giddiness, nausea, unaccountable fear and anxiety

More often than not, the disease begins abruptly, with such clear cut manifestations that the patient remembers even the hour of its onset Independent movement becomes Impossible Body temperature may rise to 38° C and on the second day to 39-40 5° C The acute stage goes on from 7 to 8, seldom 12 to 14 days The temperature falls lytically during 2-3 days, but may again rise to subfebrile level

Often there is nausea, vomiting, severe headache, especially when turning the head or eyes The pulse is slow, the occipital muscles are stiff, swallowing obstructed, tongue only partly mobile Paralysis of the neck and upper limbs may occur on the very first day Frequently observed are loss of consciousness, delirium, sleepiness Children may have epileptic attacks and convulsions In particularly severe cases death occurs in the acute period Lethality may be 5 to 20 per cent and more

The disease is known to assume a moderate, abortive or mild course Sequelae include residual muscular paralyses of the neck and arms (A G Panov, A N Shapoval et al) The farther north, the less severe is the course of the disease, with increasing incidence of *formes frustes*, which may sometimes complicate or confuse diagnosis

Distribution Besides a number of areas in the taiga and forest biomes of the U S S R, foci of tick-borne encephalitis are met in North Eastern China, Czechoslovakia, Poland, Yugoslavia, Bulgaria, Hungary, etc There are also indications of similar diseases in the tropical forests of India affecting monkeys The causative virus has been isolated from *Haemaphysalis* (Work, 1958)

HISTORY OF RESEARCH

As a specific neuroinfection, tick-borne encephalitis was first recorded in the Far East by A G Panov (1934) At the beginning of development work in the taiga, the U S S R People's Commissariat of Public Health was notified of the appearance of severe cerebral diseases which occurred among lumbermen and other people entering the taiga Immediately, expeditions were organised under the auspices of the said Commissariat and VIEM (All-Union Institute of Experimental Medicine)

The first expeditions included specialists in various fields—virologists, parasitologists, zoologists, neuropathologists and epidemiologists Such a selection of personnel ensured a speedy solution (only in 3 years time) of all basic problems regarding the causative agent, vectors, exposure and many others The data obtained in the first years (beginning with 1938) provided a scientific basis for developing general tick control measures and a number of individual and public safeguards for practical implementation by the public health service In the same years, specific prophylactic means i e, vaccine and serum, were elaborated

The research work on tick borne encephalitis and other diseases, played a major part in the origin of the theory on the natural focality of human diseases, initiated by Y N Pavlovsky (1938) and successfully developed with the participation of numerous disciples, followers and collaborators

Subsequent research on tick borne encephalitis furnished data for establishing the prevalence of its natural foci in extensive woodland areas of the Soviet Union

DISEASES SIMILAR TO TICK BORNE ENCEPHALITIS

1. Two-Wave Tick-Borne Encephalitis

Synonyms *Milk fever, two wave milk fever, two wave meningo encephalitis*

The disease ensues with a two wave temperature rise after ingestion of raw goat milk or derivatives of the latter (cheese, curds) During the season when the ticks parasitise animals, the causative agent invades goats, whose milk becomes a source of infection for man The incubation period in these cases lasts from 9 to 14 days Contagion is likewise possible from bites by *Ixodes persulcatus* and *Ixodes ricinus*, when incubation lasts from 5 to 7 days The onset is abrupt, with headaches, nausea and vomiting

High temperature persists for 5 to 8 days, following which the patient feels almost well After that comes a second temperature rise, which continues for 10 days The second attack is usually more severe, with symptoms of cerebral lesion

The seasonal prevalence of the disease is similar to that of tick borne encephalitis (A A Smorodintsev, M P Chumakov et al) The source of infection lies in natural foci

Many authors consider tick borne encephalitis and two-wave fever to be synonymous, the clinical distinctions depending on the routes of infection

2. Omsk Hemorrhagic Fever

The course includes one or two waves of high temperature continuing from 4 to 12 days Typical symptoms are nasal, uterine, gingival, and intrarenal hemorrhage, hemorrhagic rash on the skin, and in some cases bronchial pneumonia

The causative agent is a virus having much in common with the pathogens of tick borne encephalitis and two-wave meningo encephalitis The vectors are the ixodid ticks *Dermacentor pictus* and *Dermacentor marginatus* (M P Chumakov, G I Netsky and T N Zakorkina et al)

CAUSATIVE AGENT OF TICK BORNE ENCEPHALITIS

The causative agent is a filtrable virus 20 to 30 m μ in size, discovered by Soviet scientists (L A Zilber, E N Levkovich, A K Shubladze, M P Chumakov, V D Solovyov, 1937) Its presence in the blood may be revealed in the first 6 to 8 days of infection The virus reproduces and concentrates in the nerve tissue

Specific virucidal antibodies neutralising the virus develop in the blood of post convalescents on the 15th day from onset The former s presence may be detected 12 or even more years after infection Antibodies have also been found in people never afflicted with the disease, but living in the foci and exposed to tick bites The explanation to this fact was given in experimental works by Y N Pavlovsky and V D Solovyov (1940) The virus is successfully grown on chicken embryo and tissue cultures A common reactor is the white mouse

VECTORS

The chief vectors of tick borne encephalitis in the U S S R are *Ixodes persulcatus* P Sch and *Ixodes ricinus* L In certain southern areas of the Far East the principal vectors are ticks of the genus *Haemaphysalis* The vectors are sometimes spread over considerable areas, both adults and nymphs and even larvae attacking man In some habitats (small enclaves of steppe surrounded by woodland particularly on south facing foothills, on the border between woodland and steppe, or in woodland copses in the steppe) *D silvarum* Ol (Figs 2 4) may also have epidemiological importance

Vector Distribution

Ixodes persulcatus occurs throughout the southern part of the taiga, from the Baltic Sea to the Pacific Ocean, including South Kamchatka, Sakhalin, the Kuril Islands, and the mountain taiga of the Far East to the south of the river Amur The northward spread of *Ixodes persulcatus* is limited by climatic factors (summer warmth during vegetative period, low winter temperatures, permanently frozen soil) Hence in the more favourable climatic conditions of Europe, in the north west of the U S S R, the northern boundary of its range passes through the northern taiga (63° N Lat), gradually descending southward into the temperate taiga of the eastern part of the country (62° N Lat), Western Siberia (61° N Lat) and southern taiga of the Far East (53° N Lat) In the south of its range it occurs in European mixed forests, in pure hardwoods (54° N Lat) *and in the mountain taiga of the Tien Shan* (42° N Lat), Altai and Savan mountains (G V Serdyukova, 1956)

I persulcatus is unevenly distributed through the forests Its numbers are largest in woodland biotopes harbouring the hosts of adult ticks—wild ungulates—or on the grazing grounds of domestic cattle The usual habitat of these ticks *are softwoods with considerable inclusions of small leaved* hardwood species The ticks concentrate near forest trails roadsides clearings watering places of ungulates and *other places frequented by the hosts of adult ticks* (G V Serdyukova 1956)

I persulcatus begins to attack its hosts early in spring after snow thaw The first ticks usually appear on well sunned forest outskirts clearings and southward facing

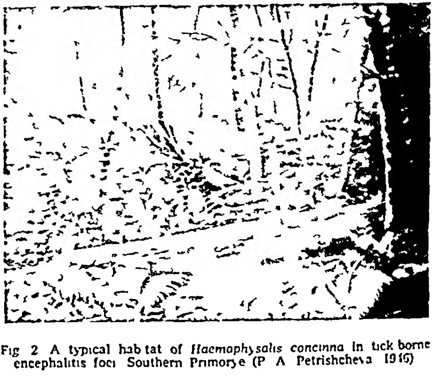

Fig 2 A typical habitat of *Haemophysalis concinna* in tick borne encephalitis foci Southern Primorye (P A Petrishcheva 1949)

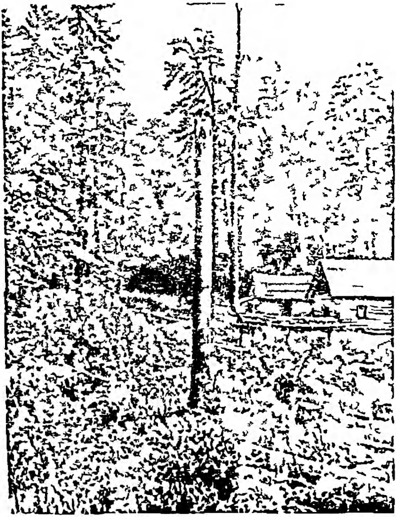

Fig 3 First buildings in Sikhote Alin taiga In such conditions *Ixodes persulcatus* often penetrates into dwellings attacking man (V E Sidorov 1957)

hill sides All three active stages prey in the first half of the warm season Adult specimens are particularly numerous in the end of May and the beginning of June, comparatively seldom attacking their hosts in the beginning of July The larvae and nymphs may also occur in notable

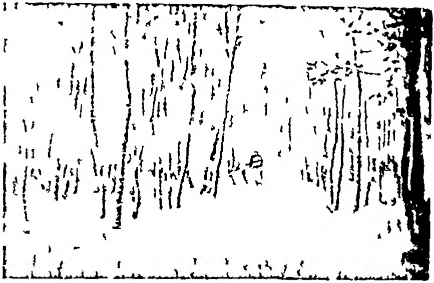

Fig 4 A birch grove early in spring (beginning of foliation) virgin land Kustanai region Focus of tick borne encephalitis with incidence of *Ixodes persulcatus* (P A Petrishcheva 1954)

quantities on small vertebrates in the end of summer In the southern boreal taiga *the development cycle covers* three years with winter diapauses in the larval and nymphal stages With a prolonged vegetational period (as in the Ussuri forests) part of the tick population completes *its development in two years, owing to omission of the* winter diapause from the nymphal stage of the cycle whereby, after moulting the nymphs may complete their feeding in the first half of summer The ticks can hibernate *in any of their active phases (G V Serdyukova 1956)*

In the European U S S R, *I ricinus* ranges from Karelia to the southern state boundary, and from the country s western borders to the eastern banks of the Volga Its *habitats occur in mixed forests (hardwood softwood)* hard woods and scrub biotopes Occasionally, it is found in number in the woodland biotopes of the elk in combined grassland and scrub communities—the favourite breeding grounds of hedgehogs, and on the forest and scrub covered grazing grounds of domestic cattle (G V Serdyukova, 1956) The ticks attack their hosts all through the warm season In the southern U S S R the seasonal preying curve of adult ticks shows two peaks, the higher of which is in May June, and the lower in August September In the north of their range, the quantity of adult ticks reaches maximum once in a season—in July August, while the number of larvae and nymphs is highest in the first half of summer The development of each stage takes a year, hence, the life cycle is completed in four years In the south the winter diapause is omitted from the oval stage, and the cycle, therefore, terminates in three years

Natural foci of two wave meningo encephalitis (European or western varieties) are associated with the incidence of *I ricinus*, whose north western range partly coincides with that of *I persulcatus* In the European part of the country there are foci of tick borne encephalitis containing both *I persulcatus* and *I ricinus*, with a prevalence of the former The range of *D silvarum* covers Kemerovo Region, Eastern Altai, Eastern Siberia, Transbaikalia and the Far East to the south of the river Amur In the Kuznets Basin the northern boundary of its distribution coincides with the northern margin of the forest steppe, and the western with the upper reaches of the river Ob (V M Popov, 1955) In Touva it has been revealed only in the alpine taiga zone

The beginning of activity of adult ticks in the south of the Primorye Territory is mid March, the end in October The seasonal preying curve shows two peaks, the number of ticks in spring (May) being considerably greater than in autumn The larvae occur *en masse* in the first half of summer, the nymphs—in July August In winter, the males find refuge on wild ungulates The life cycle is annual, with a winter diapause in the adult stage (G V Serdyukova, 1956)

The range of *Haemaphysalis concinna* is intermittent In the West, this species is widespread in the hardwoods of Europe, the Crimea, the Caucasus and the Far East The extreme north western limit of its discovery in the U S S R is in south western Byelorussia In the Far East, the tick does not occur further north than 51° N Lat Between the eastern and western regions of its distribution the tick is occasionally found in mountain forests with admixtures of small leaved varieties in such areas as Uzbekistan Kirghizia, the Savan Mountains and Western and Eastern Siberia In the Far East mature forms attack their hosts from the beginning of April to the first decade of Octobe Maximum activity is in June (Z M Zhmayeva 1941 N M Moiseyenko 1954 et al) The greatest activity of the larvae is observed in June July, and of nymphs in July August Males occur on wild animals in November, January and February In Transcaucasia mature ticks feed from March to September with a maximum in June, the larvae being active from March to October, and the nymphs from April to November (B I Pomerantsev, N V Matikashvili, 1940)

Isolation of the Virus from Ticks

The occurrence in nature of ticks of the species *I persulcatus*, *D silvarum* and *H concinna* spontaneously infected with the virus of tick borne encephalitis was first established in the Far East in 1937 During a blood meal the ticks transmitted the virus to a mouse (A N Skrinnik and N V Ryzhov) The results of expeditions conducted in 1938 and 1939 (A K Shubladze, G V Serdyukova N V Ryzhov, A N Skrinnik, A V Kozlova, V D Solovyov) revealed the occurrence of spontaneously infected ticks in all localities with recorded cases of the disease Most of the virus strains were isolated from hungry and engorged males and females of the species *I persulcatus*

The success of isolation depends on the season and specific features of tick biotopes (Y N Pavlovsky, 1947) and may vary annually In the Suputinsk forest reserve (*Ussuri*

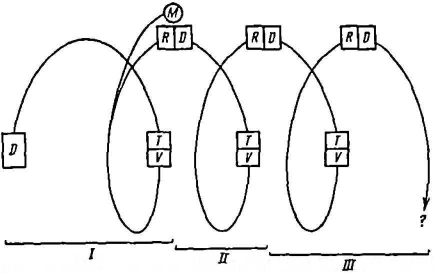

Fig 5 Circulation of tick-borne encephalitis From the donor (D) the virus is transmitted to the recipient (R) which during virusemia may serve as donor for fresh ticks (T) which become vectors transmitting the virus to new recipients $\boxed{R \mid D}$ denotes one organism, $\boxed{\frac{T}{V}}$ stands for one and the same tick specimen

A tick may convey the virus to man (M) but here practically the chain will end, the human body presenting a "blind alley" for the virus

I, II, III denote the successive stages of virus circulation

region) 27 strains of the virus were isolated in the first half of the summer of 1939 (N. V. Ryzhov, A V. Kozlova) By the end of the same summer, however, not a single strain of the virus could be revealed (V. D Solovyov), while in the first half of the next summer (1940) 6 strains were obtained (I. A. Moskvin). According to V. M. Popov et al (1957), in the Tomsk focus the virus is more often and easily obtained from ticks in early spring and the beginning of summer, and from animals in the second half of summer and in autumn. As reported, the virus of encephalitis may spend the winter in the body of *I. persulcatus* (E. N. Levkovich, A N. Skrinnik, 1940).

All the above named tick species are trihostal, i e , in each stage of its development the tick feeds on a fresh host. In each species, all three metacyclic stages have been found to transmit the virus—a phenomenon called trans stage transmission (A K Shubladze, G V Serdyukova 1939, A N Skrinnik and N V Ryzhov, 1941, A V Kozlova and V D Solovyov, 1941, M P Chumakov, 1939, 1944 A L Dumina, 1957, et al) The *transmission of the virus* from the female to its progeny (transovarial transmission) has also been proved for *I persulcatus* (A K Shubladze *and G V Serdyukova, 1939, M P Chumakov, 1944)* and *H concinna* (A V Kozlova and V D Solovyov, 1941) Generation to generation transmission of the virus in *I persulcatus* has been established as well (M P Chumakov, 1944)

In subsequent years, strains of the virus in question were repeatedly isolated from the said tick species by many authors and in various foci Recently, strains of the virus were obtained in Siberia from ticks of the species *I plumbeus* Leach , collected from sand martins (Y V Fyodorov and M K Tyushnyakova 1958) and *D marginatus* (M K Tyushnyakova, V M Popov et al , 1959) and in the Primorye territory of the Far East from *H japonica douglasi* (L V Tatarinova and N P Belikova, 1958) The circulation of the virus in the bodies of *I persulcatus* and *H concinna* has been studied by Y N Pavlovsky and V D Solovyov (1940 1941) An active form of the virus was revealed in the blood, ovary, nerve ganglion and salivary glands of *I persulcatus* A less active strain was found in the salivary glands stomach and ovary of *H concinna* M K Tyushnyakova (1955) notes the annual *fluctuations* of *infection in I persulcatus* in the south of the Tomsk focus of encephalitis, which was 3 per cent in 1950 1 6 in 1951, 3 2 in 1952 and 2 1 per cent in 1953 The highest percentage of infected ticks was revealed *in* southern, and the lowest in northern areas

Experimentally infected ticks of the species *I persulcatus* reveal the virus irregularly, which becomes more manifest with the progress of metamorphosis and in subsequent generations The ability to receive the virus and maintain it in subsequent metacyclic stages depends on the degree of *virusemia in the donor's blood* When ticks are fed individually on diseased white mice, not all the former develop infection (e g , 2 nymphs out of 18, and 3 females out of 13) Transovarial transmission is likewise uneven, the virus not being transmitted to the entire progeny Not all the tick specimens ingesting the virus during blood meals will retain it further When non infected and infected ticks are fed simultaneously, the former will receive the virus easier if fed on suckling animals On adult animals, *the ticks are not infected throughout On immune animals* and their suckling progeny, infection does not occur at all (A L Dumina, 1957)

Studies on virus incidence in ticks are the first and most customary procedure in determining the epidemiological importance of any focus of tick borne encephalitis As a rule, the ticks reveal the virus more frequently than do mammals or birds Hence, when investigating foci, the usual practice is to test ticks for incidence of the virus, and vertebrates—for virucidal antibodies The virus of tick-borne encephalitis was isolated from *Gamasidae* (E N *Levkovich* and A A *Tagiltsev, 1957) and fleas (A K Shubladze* and N I Kalabukhov, 1944, Y V Fyodorov, M K Tyushnyakova and N I Igolkin, 1959)

Determination of Tick Species and Their Importance in Foci of Tick-Borne Encephalitis

When determining the tick fauna of a given focus, the primary task is collection of live ticks from the taiga area under survey, which may be either completely virgin or visited by people engaged in various development work (road building, timber felling, erection of temporary or permanent structures) or other economic activities (cattle grazing, vegetable gardening, ploughing, haymaking, etc) The same applies to forests previously affected by man

The purposes of tick collection may be different, viz, identification of the local tick fauna and study of its annual metacyclic dynamics, determination of preying seasons and the fullest possible identification of the range of its hosts Ticks removed from the clothing and bodies of people are collected and stored separately Live ticks are used to determine the duration of different metacyclic stages, larvae and nymphs being reared to the stage of imago for the purpose of correct classification

The ticks are tested for possible spontaneous infection and also experimentally infected to assess their potential importance as vectors in natural environments In the laboratory, they are studied for stage-to stage and transovarial virus transmission, the latter to be observed on a series of descendant tick generations, since up to now the phenomenon has been clarified only for two successive generations Live ticks are required for studies on various aspects of their vital activity, as well as for laboratory and field investigations on the efficiency of different acaricides, etc, etc

Ixodid ticks are collected while hungry, chiefly on open spaces in nature and in the vicinity of man It is likewise necessary to collect ticks attached to their hosts for convenience choosing those which have climbed on the host but have not yet selected a spot for suction The majority of ticks found on animals are usually fully or semi engorged and, consequently, fall off the host and may be found on the ground or in sheltered abodes where they crawl to lay eggs In winter time, in the south ticks may be found among the fur of goats and camels where it is warmer than outside Hunters should be asked to examine killed hares or other animals and collect all ticks discovered

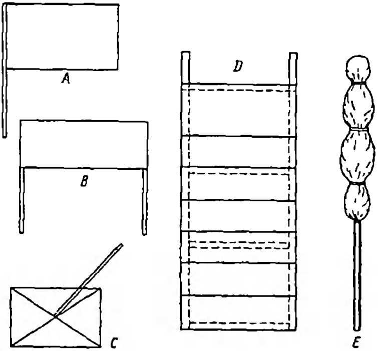

Fig 6 Devices used for collection of ixodid ticks
A—flag, B—cloth hauler; C—scraper, D—screen, E—probe

In foci of tick-borne encephalitis, usually associated with taiga of definite botanical composition or forests affected by age-long human activity, special methods of collection should be used, some of which are described hereunder.

Mature ticks are collected in nature by means of a flag, cloth hauler or scraper. A collecting flag is made of flannel 100×60 cm in size which is secured to a stick (Fig. 6). The cloth hauler presents a piece of flannel 125×60 cm in size, into whose narrow hems wooden sticks or wire rods are inserted. The cloth is tied to a 50 cm stick by means of a string. A scraper is made of a piece of plywood 50×40 cm in size covered with flannel and diagonally fixed to a handle (B. I. Pomerantsev and G. V. Serdyukova) A V Mishin employs a hauling cloth 120×60 cm in size with its narrower hem secured to a limewood fork The same implements and cloth dimensions should be used throughout the entire season of observation

The surveyor slowly hauls the device over the under growth on his right hand side or in front The collected ticks should be taken off every 2 or 5 minutes At longer intervals between inspections part of the earlier collected ticks may be lost especially when passing the device through the rougher parts of plants and shrubbery

The abundance of ticks in various habitats is determined by the number of specimens collected from the flag per hour (flag hour) or removed from the collector himself (man hour) or from 1 kilometre of the distance covered in a unit of time In the latter case a pedometer is used previously measuring the distance normally covered by the collector at a single stride On rough or deadwood littered terrain the ticks are counted by flag or man hours which however provides but a very rough estimate

Tick counts should be accompanied by meteorological observations conducted along the route Air temperature and relative humidity should be measured at 2 cm 50 cm (grass level) and 120 cm above ground Litter temperature should be taken at a depth of 1 to 5 cm Relative humidity may be measured with an August or Assmann psychro meter If possible light intensity (in luxes) is measured at litter level (under the foliage or beneath clearings)

The vertical distribution of ticks in scrub growth is measured with a screen made of poles two metres long fixed together transversely with 75 cm bars This frame is covered with white cloth lined into transverse stripes 25 cm high Two workers haul the screen through the grass or low shrubbery in a position as close to vertical as possible After every fifteen or twenty paces the screen is examined noting the height of tick attachment (B I Pomerantsev G V Serdyukova 1939) All the data on ticks are entered in a field journal (see record forms applied)

Field Journal for Tick Activity Data Collected on Route

Route No	Date and hour of survey	Distance from route starting point and number of ticks per 20 m											Total number of ticks including those from collector
		20	0	60	80	100	120	140	160	180	200	etc	

Record of Meteorological Data

Date of measurement

Meter readings	Hour of measurement	Litter at depth of 1.5 cm	At 2 cm above ground	At grass level (50 cm)	At 120 cm above ground	Note
Thermometer Psychrometer Luxmeter						

Summary Record of Tick Activity on Fixed Routes[1]

Number of ticks													Weather conditions						
													Temperature				Relative humidity		
Date	Route No	Hours of survey	Total	Males	Females	Larvae	Nymphs					etc	Litter	At 2 cm above ground	Above grass	At 120 cm above ground	At 2 cm above ground	Above grass	At 120 cm above ground

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[1] Data obtained from field records

Hungry larvae and nymphs poised on the tips of grass blades are gathered by means of a scraper dragged forcefully along with its narrow edge to the ground (covered with low grass), periodically collecting the adhering larvae and nymphs On more open forest glades, a towel on two handles is smoothly drawn over the growth

A large amount of pre imago forms, especially larvae, may be collected on a flag slowly hauled around stumps This circumstance indicates the possible occurrence of egg deposits along the root tunnels and in the pockets between the roots proper In the forests of the Leningrad region, larvae have been found in profusion around stumps in the end of May (P A Petrishcheva)

For regular long term observations of tick behaviour, quadrangular plots with vegetation measuring from 5X5 to 100X100 m and more are isolated by planks or ditches The ticks are let out on the plots, observing their dispersal In the Suputinsk forest reserve, *I persulcatus* were proved to be aware of human scent The ticks concentrated at the side of the plot facing a pathway frequented by people On experimental plots, the number of ticks taken from identical habitats may be set at will, systematically observing their behaviour, i e, metamorphosis, length of starvation in various stages, wintering, migrations, variations of activity during the season or day and night under different weather conditions, etc For each of these purposes separate plots of different size and character are chosen It is important to note the local situation in regard to direct and dispersed sunlight, as well as features of forest litter humidity, warmth, etc In alpine regions, simultaneous observations should be made on northern and southern slopes In such cases the time difference in the beginning of tick activity may comprise from 30 to 45 days If the area under survey contains rodent burrows, the latter s content should be taken and inspected for ixodid ticks Of special interest are snow covered nests of rodents abound

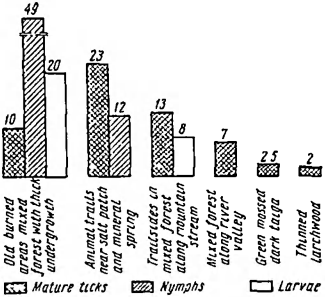

Fig 7 Comparative population of *Ixodes persulcatus* in neighbourhood of Vangow township southern flanks of Sikhote Alin (P A Petrishcheva 1946)

ing in *Gamasidae* which should be collected shortly after spring thaw

In the taiga, route collections of hungry ixodid ticks are conducted in areas passed by animals on their way to watering places or frequented by people Observations by B I Pomerantsev and G V Serdyukova in the Suputinsk reserve showed that even on the trails proper the distribution of ticks may vary widely When collections were extended deeper into the forest, outside the trail, the number of ticks was observed to fall rapidly, to the extent of complete disappearance This frequently occurred at a distance of only three to five metres from the road or trail side Heaps of deadwood or timberwork refuse harbouring small animals should be examined with special care A notable increase in the number of ticks may be observed in the resting places of larger game, usually de-

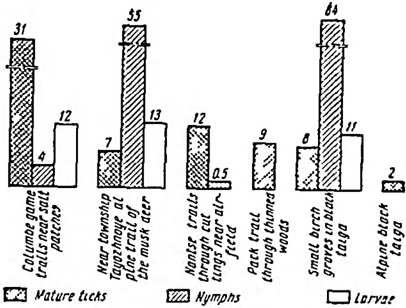

Fig 8 Comparative population of *Ixodes persulcatus* in Sikhote Alin Mountains Krasnoarmeisk district Primorye territory Figures over columns denote collections per flag hour (P A Petrishcheva 1957)

tected by the depressed growth Abundant collections were made near watering places and especially along game trails leading to salt patches regularly visited by large game

Parallel observations are made to clarify the features of habitats in nature and on terrain modified by human activity Investigations of various habitats for tick infestment afford a vivid picture of the mosaic of tick distribution permitting the establishment of localities with the greatest hazards for man (Figs 7 9)

Data characterising the foci of tick borne encephalitis are obtained during geobotanical surveys of plant communities noting the geographic location relief and types of soil An example of such surveys is the work of the botanist Pisvaukova carried out near Obor Khabarovsk territory In 1941 she singled out and described individual

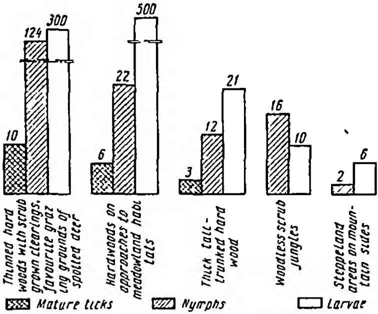

Fig. 9 Comparative population of ticks gen. *Haemaphysalis* on an island in the Sea of Japan. Figures denote collections per *man-hour* (P. A Petrishcheva, 1957)

areas in old cedar—hardwood communities, valley- and estuary-type hardwoods, larchwoods with a ground covering of sphagnum, and mixed larch- and birchwoods with a covering of *Calamagrostis.* All these areas differ in microclimate, none of them being tick-infested throughout. Tick foci in the Novgorod region were described by B. I. Pomerantsev (1935).

Observations on the seasonal changes in the population and activity of imago forms should include counts to be carried out not less than once in five days. Counts should be made at the same hours, at the time when the ticks are most active, i.e., from 10 to 12. a.m. (but not earlier, owing to heavy dew) and 5 to 8 p.m. (the hours of tick activity vary locally). Ticks from domestic animals are collected at control points every 5 to 7 days, always noting the character of the pasture and the length of grazing time.

It is advisable likewise to count the ticks on the dogs which always accompany herds and are subject to mass tick attacks

The quantity of ticks on pastures should be noted together with the intensity and time of grazing Frequently, the same grazing grounds are visited by both domestic and wild animals (e g, spotted deer in the Far East), which should be made particular note of Domestic animals sustain an enormous amount of ticks, whence the number of ticks on pastures is many times more than the amount in remoter parts of the forest where the only hosts of ticks are wild animals Surveys of control pastures are continued throughout the warm season, thus following up the ticks life cycle from their earliest emergence until total disappearance

For assessment of diurnal and nocturnal activity, not less than 8 tick counts are made during the day and night with the use of the same collection techniques Such counts should be repeated from 8 to 10 times per season on control plots specially chosen for the purpose The methods employed for capturing animals as well as tick-collecting techniques are presented in the subchapter on ixodid ticks as vectors of rickettsial diseases Immature ticks may be collected *in nature* by *means of live bait—white mice*, wild *Muridae*, hedgehogs The animals are put into small cages made of metal netting R R Sharipova (1957) recommends putting white mice into plywood cages with a supply of food, providing free exit and entry

It is advisable to present the seasonal dynamics of tick activity in its relation to the phenological plant spectrum Phenological phenomena in the flora should be noted on plants most typical for the given area Phenomena to be noted include unfolding of buds, appearance of leaves and flower buds, blossoming, fruiting The indicators to be used are birch, aspen prickwood lilac bird cherry, rowan-tree, acacia, hazel dog rose, currant, raspberry, etc

For experimental observations on the development time of separate stages in various biotopes, the ticks are maintained in 60×40 mm cages of wire netting (mesh—1 mm) or capron mill gauze Tick hibernation is best observed in mill gauze covered wooden boxes 4×10×2 cm in size, placing them in various biotopes at various litter depth Such boxes will better last through the winter in the litter, the ticks contained endure conditions corresponding to their natural environment At the sites chosen for the cages it is necessary to measure the depth of the snow Tick migrations may be studied by marking the arthropods' backs with enamel paint

The number of ticks in one and the same place varies annually, depending on climatic factors and the abundance of hosts Hence, the studies should be repeated for several different years

The methods employed for the study of ticks should be chosen with regard to the conditions of work In route type expeditions the work is chiefly restricted to collection of live specimens and observations en route In expeditions of more stationary character certain laboratory work is possible The full scale study programme may be carried out at permanent establishments set up in regions and areas harbouring natural or man affected foci of tick-borne encephalitis

An all round survey of a focus should include the following virological studies (1) isolation of the pathogen from patients, ticks and wild animals, (2) testing of small wild fauna and large domestic animals for complement fixing and virus neutralising antibodies

Investigations on spontaneous tick infection are carried out by feeding the latter on white mice as well as preparing suspensions of tick viscera for subsequent inoculation of white mice To assess the percentage of infected ticks in different biotopes, 10 mature ticks should be taken per test, since otherwise the results obtained from different foci of the U S S R cannot be compared In primary investigations of a locality for infected ticks, up to 30 hungry adults and 500 nymphs and larvae should be taken per test

IMPORTANCE OF VERTEBRATES IN FOCI OF TICK BORNE ENCEPHALITIS

Vertebrate hosts According to summary data supplied by I G Galuzo (1950), G V Serdyukova (1956) et al among the animals inhabiting the U S S R which serve as hosts for ticks in foci of tick-borne encephalitis, there are 253 species representing three classes of vertebrates, viz 103 species of mammals, 141 birds and 9 reptiles The total number of hosts varies considerably for different species of ticks, comprising 194 for *I persulcatus*, 111 for *I ricinus* 70 for *H concinna*, 56 for *D silvarum*, and 21 for *H japonica*

Among the multitude of animal host species inhabiting the foci, the importance of different species is far from equal, and at present may be assessed by relative data on tick infestment, the so-called abundance index (mean number of ticks per inspected animal), intensive infestment (mean number of ticks per animal infested) extensive infestment (percentage of tick infested animals among the total inspected population) Since practically any animal may become a host, the most numerous and mobile among them are likely to be most important in this respect Accordingly, principal, supplementary and secondary hosts may be distinguished for each metacyclic stage The principal hosts in a given focus are the species with a higher degree of infestment, occurring in comparatively large and stable numbers Species with a high degree of infestment but irregular in number are referred to as supplementary hosts, and those with permanently low infestment are known as secondary hosts By virtue of their permanent presence, the principal hosts ensure the stable existence of ticks in various parts of natural foci of tick borne encephalitis The periodical mass appearance of supplementary and secondary hosts leads to expansion of the tick population and promotes their wide spread through the focal area

However, in each given focus, the ticks in the course of their metamorphosis have to do not with separate species, but with a definite assortment of hosts The importance of individual host combinations may vary both seasonally and annually Thus, the bank vole shrew combination providing for the existence of ticks in their first stage, occurs in all foci throughout the U S S R At the same time, the numbers of either species are known to vary from year to year These quantitative variations are usually asynchronous, but the permanent presence of either of the species *ensures the survival of ticks in the foci The nymphal* host combinations may also be permanent (birds, hedgehogs) or variable (squirrel, hare, chipmunk, etc) in content The total nymph population depends on the number of nurtured larvae, i e , indirectly, on variations in the number of small mammals

The importance of different species in sustaining ticks *is unequal The most typical host species among mammals* are the bank vole (g *Clethrionomys*), shrews (g *Sorex*), mice (g *Apodemus*), northern birch mouse (*Sicista betulina*), chipmunk (*Eutamias sibiricus*), hedgehog (*Erinaceus europaeus*) and, apparently, all woodland species of carnivores and ungulates Among birds, the most important are biological groups containing species which collect their *food from the ground (tree pipit, bunting, thrush, Gallinaceae*, starling, jay, chaffinch and certain others) However, the infestment indices and, accordingly, the importance of these bird groups in various foci, differ considerably In European foci their importance in maintaining ticks is less, being appreciable only in years with high tick populations It should be remembered that owing to their high mobility, birds are of great importance in disseminating ticks through an area

In some foci, the open space species, i e, so called meadowland and field forms, turn out to be the most important hosts, which is mostly manifest in regions with large areas of ploughland or meadows emerging in the place of felled forests Being unstable in number, these species may occasionally become the most important hosts This happens particularly often in European foci with the common vole (*Microtus arvalis*), in Western Siberia with the narrow skulled vole (*Microtus gregalis*), common hamster (*Cricetus cricetus*), in the Far East with the reed vole (*Microtus fortis*), the harvest mouse (*Micromys minutus*) and almost everywhere with the striped field mouse (*Apodemus agrarius*)

Individual metacyclic stages are most frequently encountered on definite animals, larvae—on small mammals and birds, nymphs—on medium sized mammals and birds imago—on large vertebrates Occasionally, in absence of the habitual hosts, the ticks may suck blood from other animals For example, nymphs may occur on small mammals, and adults on mammals of medium size Thus, the highest incidence and relatively highest infestment rates are mostly associated with hares (g *Lepus*) hedgehogs (*Erinaceus europaeus*) and less with squirrels (*Sciurus vulgaris*) and chipmunks (*Eutamias sibiricus*) The aforesaid phenomenon of host substitution is of major importance in the maintenance of natural foci

Vertebrate vectors of tick borne encephalitis At present spontaneous infection with the virus of tick borne encephalitis has been revealed in 24 species of mammals and 14 birds (see table)

In all foci certain spontaneously infected mammals and birds have been demonstrated to be the principal hosts of ticks The greatest number of virus strains has been isolated from bank voles, shrews yellow necked field mice forest mice and chipmunks and among birds—from pipits wood hens, buntings and thrushes The number of spontaneously infected vertebrates increases from west to east

Spontaneous Infection and Susceptibility[1] to the Virus of Tick Borne Encephalitis in Vertebrates in the U S S R

Species	Region in which spontaneous infection was found	Author and year	Susceptibility
1	2	3	4
Common hedgehog (*Erinaceus europaeus*)	Far East	V D Solovyov 1938	S
Moger s mole (*Mogera robusta*)	Far East	V D Solovyov 1938	?
Common shrew (*Sorex araneus*)	Perm region	A L Dumina 1958	?
	Tomsk region	M K Tyushnyakova 1954	
	Altai	A A Pcholkina 1957	
Common squirrel (*Sciurus vulgaris*)	Sverdlovsk region	M P Chumakov 1939	N
Chipmunk (*Eutamias sibiricus*)	Sverdlovsk region	M P Chumakov 1939	
	Tomsk region	M K Tyushnyakova 1955	L
	Far East	V D Solovyov 1938	
Forest dormouse (*Dyromys nitedula*)	Tatar A S S R	G Kh Gilmanova 1958	?
Northern birch mouse (*Sicista betulina*)	Tomsk region	M K Tyushnyakova 1954	?
Field mouse (*Apodemus agrarius*)	Tatar A S S R	G Kh Gilmanova 1958	L
	Far East	A N Sotnikova and I M Ambarnikov 1956	
Common forest mouse (*Apodemus sylvaticus*)	Leningrad region	E N Levkovich 1942	
	Velikiye Luki region	A K Shubladze 1942	
	Perm region	V D Solovyov 1941	?
	Kuibyshev region	L B Popova 1954	
	Tatar A S S R	G Kh Gilmanova 1958	
	Kazakh S S R	I G Galuzo 1942	

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[1] Data on susceptibility are given only for species revealing spontaneous infection with the virus of tick borne encephalitis Symbols S—susceptible L—low susceptibility N—non susceptible ?—susceptibility not established

(Continued)

Species	Region in which spontaneous infection was found	Author and year	Susceptibility
1	2	3	4
Large Japanese field mouse (*Apodemus speciosus*)	Altai Far East	A. A. Pcholkina, 1957 A. G. Panov, 1948	[illegible]
Yellow necked field mouse (*Apodemus flavicollis*)	Kuibyshev region	V. D. Solovyov, 1940	1
Common hamster (*Cricetus cricetus*)	Tomsk region	I. D. Ronzhina, 1946	2
Bank vole (*Clethrionomys glareolus*)	Byelorussian S.S.R. Perm region Bashkir A.S.S.R. Tatar A.S.S.R. Omsk region	M. P. Chumakov, 1940 V. D. Solovyov, 1940 G. Kh. Gilmanova, 1956 G. Kh. Gilmanova, 1958 T. V. Zakorkina, 1958	2
Northern red backed vole (*Clethrionomys rutilus*)	Perm region Omsk region Tomsk region Altai	V. D. Solovyov, 1940 T. V. Zakorkina, 1958 M. K. Tyushnyakova, 1954 A. A. Pcholkina, 1957	2
Large toothed red backed vole (*Clethrionomys rufocanus*)	Altai Perm region Far East	A. A. Pcholkina, 1957 A. V. Petrova, 1941 V. D. Solovyov, 1938	4
Common vole (*Microtus arvalis*)	Tomsk region	M. K. Tyushnyakova, 1952	5
Field vole (*Microtus agrestis*)	Tomsk region	M. K. Tyushnyakova, 1954	3
Root vole (*Microtus oeconomus*)	Altai	A. A. Pcholkina, 1957	3
Narrow skulled vole (*Microtus gregalis*)	Tomsk region	I. D. Ronzhina, 1946	3
Altai pika (*Ochotona alpina*)	Altai Krasnoyarsk territory	A. A. Pcholkina, 1957 T. V. Zakorkina, 1958	3
White hare (*Lepus timidus*)	Sverdlovsk region	M. P. Chumakov, 1939	[illegible]

(Continued)

Species	Region in which spontaneous infection was found	Author and year	Susceptibility
1	2	3	4
Class Birds (Aves)			
Wood hen (*Tetrastes bonasia*)	Altai Krasnoyarsk territory Far East	A A Pcholkina 1957 T N Zakorkina 1958 I A Moskvin 1940	?
Cuckoo (*Cuculus canoris*)	Altai	A A Pcholkina 1957	?
Common bunting (*Emberiza citrinella*)	Tomsk region	Y V Fyodorov 1956	L
Yellow necked bunting (*Emberiza elegans*)	Far East	I A Moskvin 1940	?
Chaffinch (*Fringilla coelebs*)	Tomsk region	Y V Fyodorov 1956	?
Tree pipit (*Anthus trivialis*)	Tatar A S S R Tomsk region	G Kh Gilmanova 1958 Y V Fyodorov 1956	?
Indian tree pipit (*Anthus hodgsoni*)	Krasnoyarsk territory	T N Zakorkina 1958	?
Nuthatch (*Sitta europaea*)	Far East	I A Moskvin 1940	?
Fieldfare (*Turdus pilaris*)	Tomsk region	L D Ronzhina 1946	?
Grey backed thrush (*Turdus hortulorum*)	Far East	I A Moskvin 1940	?
Black throated thrush (*Turdus rubicollis*)	Krasnoyarsk territory	T N Zakorkina 1958	?
White throated rock thrush (*Monticola gularis*)	Far East	A N Sotnikova and I M Ambarnikov	?
Golden mountain thrush (*Oreocincla dauma*)	Far East	D G Tatarinova 1957	?
Blue nightingale (*Luscinia cyane*)	Far East	I A Moskvin 1940	?

the specific assortment of the infected animals likewise changing

Apart from typical woodland species, infection has been revealed in a number of species associated with open terrain in the common and narrow-skulled voles (*M arvalis* and *M gregalis*), common hamster (*C cricetus*) and among birds, in tree pipits and Indian tree-pipits (*A trivialis* and *A hodgsoni*) In most cases, natural infection in animals was established by isolating the virus from the brain, its incidence in the blood being very rare, which indicates limited disseminating capacity and the transitory character of hostship in vertebrates The transitory importance of warm blooded vertebrates as hosts is also testified by (a) considerably lower incidence of infection in the former than in ticks (b) in some years, at mass surveys total absence of infection (in contrast to the frequent incidence of the virus in ticks) (c) later incidence of the virus in animals than in ticks

SUSCEPTIBILITY

V D Solovyov (1939, 1940, 1941, 1944), A A Smorodintsev et al (1939, 1943), I A Moskvin (1940), E N Levkovich et al (1954) E S Sarmanova et al (1956) have experimentally established varying degrees of susceptibility to the virus of tick borne encephalitis in different animals and birds (see table) Individual species were set down as susceptible when inoculation was followed by the development of obvious symptoms of the disease, the virus circulating in their blood for longer periods Low susceptibility was stated for animals with a symptomless course of the disease and short term virusemia (not more than 15 days) The non susceptible or resistant species are those in which the virus is maintained for a short length of time and does not accumulate

As evident from the table, many species peculiar to foci of tick borne encephalitis have not been studied These include mammals, such as all the species of shrews moles bank voles and birds such as pipits and thrushes etc Of the species already studied, most woodland forms appeared to be low susceptible or resistant The degree of suscep tibility did not prove to be a specific feature throughout, individual variations being revealed in different specimens of the same species Thus, hedgehogs obtained from areas endemic and non-endemic for tick borne encephalitis, react differently to inoculation with the virus (V D Solovyov) The same was revealed in such highly susceptible mammals as grey voles (*M arvalis*) in the experiments by V D Solovyov and E S Sarmanova, and in linnets (*Acanthis flammea*) by I A Moskvin

Apparently, the resistibility of different specimens towards encephalitis is determined by their individually acquired immunity Possibly, the species more frequently contacting ticks in the course of evolution, developed a respectively higher degree of immunological adaptation Otherwise, in case of high susceptibility to the disease, these species could not have survived in the focal biocenoses, and the foci themselves could hardly have proved to be stable This, probably, accounts for the fact that the animals and birds classed as low-susceptible and resistant, usually include the most typical woodland forms On the other hand, species non-typical for woodlands, but occurring therein, e g , meadowland forms (grey voles), reveal an exceedingly variable degree of individual resistance to infection

In vector ticks, contagion may take place only when the virus occurs in the peripheral blood of the donor Hence the length and intensity of virusemia are indicative of the importance of various animal species in focal biocenoses Experiments by E S Sarmanova (1948), and by the same in collaboration with A L Dumina (1956) demonstrated that both susceptible and non susceptible animals generally carry the virus in their central nervous system for longer periods than in their blood The term of virus circulation in the blood varies broadly for different animals and does not correspond to the latter's level of susceptibility These variations may range as follows (1) for susceptible species grey vole (*M arvalis*) from 3 to 8 days, hedgehog (*E europaeus*) and linnet (*Acanthis flammea*) up to 15, (2) for low-susceptible species chipmunk (*E sibiricus*) from 3 to 10 days, bunting (*E citrinella*) up to 6 days, (3) for non-susceptible species large toothed red-backed vole, up to 15 days In the blood of the susceptible reed vole the virus could be revealed during three days, while in that of the low susceptible large Japanese field mouse (*Apodemus speciosus*) it remained apparent for up to ten days (A I Drobishevskaya, 1943)

Although the virusemic terms in both groups of animals are limited (not exceeding 15 days), practically, either group may infect ticks The concentration of the virus in the blood of all animals so far studied, is quite sufficient for the ticks to receive the pathogen and maintain it through subsequent metamorphosis The terms of virusemia in natural conditions are incomparably shorter than in experimental conditions Hence, practically, the coincidence between the calendar terms of virusemia in animals' blood and the attacks of hungry ticks on virus-carrying hosts is of decisive importance

A potential factor limiting the spread of the virus may be immunisation of animals by the bites of virus carrying ticks The possibility of such immunisation has been proved experimentally (E N Levkovich and A N Skrinnik, 1941) Antibodies neutralising the virus have been found in the blood of many wild and domestic animal species (V D Solovyov, 1941, 1944)

Investigations carried out by A L Dumina (1957, 1958) in foci of the Kemerovo region, demonstrated that antibodies occur more frequently in rodents than in adult birds, which indicates greater contact of the former with infected ticks In these experiments the blood of nestlings revealed no antibodies, i e, birds are unable to passively transmit the antibodies from parents to progeny In the case of rodents, the incidence of immunity in the young depends on its occurrence among adult animals which may passively transmit the antibodies to their offspring Hence the comparatively greater possibility of dissemination of the virus among birds than among rodents Since virus circulation in a natural focus, on the one hand, proceeds together with the metamorphosis of ticks (from stage to stage and transovarially) and on the other hand, includes in its chain a large number of animals, the combined immunological effect on the said circulation proves considerable, all the more so that not all tick specimens involved transmit the virus from stage to stage and through the eggs (A L Dumina, 1954, 1957) A prerequisite for the maintenance of natural foci of tick borne encephalitis is the regular replenishment of the virus in ticks during blood meals, which, as stated earlier, is only possible with a sufficiently high percentage of non immune animals in the focus Best suited for this purpose are species and groups of species most frequently renewing their population (*Muridae*, shrews, squirrels, hares), or not transmitting their immunity to their offspring (birds) A special part in disseminating the virus over an area is played by species of animals broadly fluctuating in number and highly susceptible to tick borne encephalitis

On the other hand, because of their long contact with ticks in the respective foci, species with relatively stable populations and greater longevity, rather quickly develop immunity to the virus, so that their importance in spreading the infection becomes inconsiderable (large domestic and wild animals) An earlier suggestion that in these species the effect of their own antibodies may cause self-sterilisation, i e , elimination of the virus in the ticks which they feed, was not confirmed in the experiments of A L Dumina (1958)

Thus, taken alone, data on natural infection and susceptibility are not sufficient for assaying the part played by different warm blooded species in circulating the pathogen The basic criterion is not even the level of virusemia but the extent of immunisation of the animals which depends on the degree of contact between individual animal species and infected ticks Despite the ability of ticks to retain the virus during metamorphosis, the virus cannot be maintained in natural foci without regular replenishment from warm blooded species Hence, the leading role in spreading the virus may belong only to those species which regularly re stock the focus with non immune specimens capable of infecting and being infected by ticks

The yearly fluctuations in the number of infected ticks, exert an influence on the intensity of epidemiologic processes in various foci, i e, the basic epidemiological indices are largely determined bv the entire course of the respective epizootological processes, and in the first place, by the incidence of virus carrying ticks In consequence, all kinds of zoological and other research in foci of tick-borne encephalitis, should primarily be directed towards a single objective, viz, disclosing the regularities of the pathogen s circulation in nature depending on various environmental factors

TECHNIQUE OF ZOOLOGICAL RESEARCH IN FOCI OF TICK BORNE ENCEPHALITIS

General biological research Purpose determination of specific assortment, distribution and number of vertebrates Basic technique counting vertebrates and mapping the results on a chart of the area

The chart is drawn up on the basis of foresters' plans indicating different types of forest and other terrain Acquaintance with these will promote determination of the typical habitats occurring in the locality under survey and their relative square area As a basis for research, the chart is primarily needed for orientation on the terrain, while in the course of work it is used to draw routes, plot counting lines and areas, permitting a momentary estimate of the evenness of survey work over the area, which is imperative in the study of natural foci

In accordance with the local assortment of different habitats, a time table of research on the quantitative representation of various groups and species of vertebrates is drawn up Basically, there exist two counting techniques, viz, relative and absolute The former affords merely an idea of the comparative abundance of animals in different habitats and the relative yearly and seasonal fluctuations in their numbers Absolute counts serve to estimate the actual number of animals per unit of area and reflect *the true size of their population*

Counting small animals Relative counts of small animals inhabiting natural foci are accomplished by snaring or trapping with the respective devices set in lines, or by catching in pits Trap lines are employed for counting in the following way 25 traps are baited with standard mixtures (bread and carrots sliced into cubes and sprinkled with vegetable oil), and set at five metre intervals within the limits of a single habitat The traps are put out for one night and removed the next morning The relative population *index is estimated by the number of animals captured* per 100 traps Assessment of a habitat requires not less than 6 trap lines, or 150 trap days The procedure is applicable in counting bank voles, mice root voles and to a lesser extent, common voles and shrews

Counts with the use of pits are also carried out in the key habitats The pits are made one spade deep and wide and 50 m long The bottom and sides of the pit should be smooth Cylinders are set along the bottom of the pit at intervals of 10 m and no less than 40 cm deep When *installing the cylinders, no spaces should be left between* the latter and the sides of the pit The pits are examined twice daily, in the morning and evening The number of animals caught in a pit during 10 davs is taken as a unit Pit trapping constitutes the most important method for collecting and studying the feeding dynamics of ixodid larvae and nymphs The method is used in counting all species of voles field mice and small *Insectivora* (land shrews, water shrews, etc) It is less applicable for counting mice, the required cylinder height in their case being 70 cm

The basic shortcoming of counts employing trap lines and pits is that the amount of captured animals depends not only on the latter's number, but on their mobility, nor do the former provide an idea of the population density of small mammals The mentioned drawback is overcome by computing selective estimates for chosen sectors of the area The procedure comprises the following Wire cage-traps are staggered ten metres apart over an area of one or two hectares, each in a permanent place The traps are examined twice daily, marking the captured animals by cutting off their toes in various combinations (the right hind toes for digits, and the left for tens, the right fore toes for hundreds, and the left, respectively, for thousands) On marking, the animals are released, having previously collected their ectoparasites Further repeated captures of the same animals will provide an estimate of the number of specimens inhabiting the sector, i e , the absolute animal population A shortcoming of the method, apart from laboriousness, is that it may only be employed for rodents—mice, hamsters, bank voles, root voles, and to a lesser extent for other voles

Squirrels, chipmunks, hares, hedgehogs Squirrel populations are counted along control strips with the help of a dog The observer walks along a fixed route through the forest at an even pace of 2 or 3 km per hour and counts all squirrels barked at by the dog, noting the type of vegetation Control strips should cover not less than 1 per cent of the area of typical habitats, the routes extending for not less than 10 to 15 kilometres Counts along each route should be repeated not less than 2 or 3 times in morning hours, dry weather being an imperative The number of squirrels both met and captured is computed per unit of area (totally and by habitats), assuming the range of the dog's search to be 50 m The counting results may be affected by the efficiency of the dog, which should be known beforehand Of other methods, we may advise counting squirrel nests on control routes by a team of 2 or 3 men moving abreast with intervals of 20 to 25 metres Some idea of the squirrel population may be gained by interrogating hunters

Chipmunks in spring may also be counted on control routes with the help of a dog and whistle, and in summer and autumn by checking the number of animals leaving their nests Finally, a source of indirect information may be pelt hunting data or trappers' reports

Hare counts are usually made in winter, the animals being driven along control strips not wider than 500 m The team members delete all tracks around the plot, and start out, moving with as much noise as possible After the procedure is over, all exit tracks are counted In spring, prior to the development of grassy vegetation, hares may be counted with a dog

Studies of hedgehog population and distribution are also made with dogs on routes or sectors specially marked off for the purpose

Birds Passeriformes are counted on routes or sectors, *Gallinaceae*—only on routes One four hectare (2,000×200 m) counting ground is marked off apiece in no less than three of the most typical woodland habitats At dawn the ground is crossed by transversal routes running parallel to each other at intervals of 50 m, note being taken of the number of singing males The count is repeated every morning for four days, the mean number of males being multiplied by two, which gives an estimate of the total bird population After the end of nesting, counting routes are laid out at 20 m intervals, and all the birds met en route are counted by sight Permanent routes 2 5 or 3 kilometres long are laid in such a manner as to cross the basic woodland types in a size ratio roughly proportionate to the areas occupied by the corresponding types of forest

One hundred metres of covered route are taken as a counting unit.

Counting routes for *Gallinaceae* are covered in a single excursion, the length of a route being not less than 10 km. The birds are counted on a strip 40 m wide (20 m to each side of the route), for each type of forest separately. The total length of all routes covered should be not less than 100 km.

Carnivores and ungulates. The existing methods of counting large mammals are painstaking, the usual time for such counts being winter. In some cases it is practicable to count lairs and burrows of carnivores during the settled period of their life. Large ungulates may be counted from aircraft. The data thus obtained provide a rough estimate of the population density of large mammals on the area under survey. Apart from direct counts, some notion of the latter's number and distribution may be obtained by certain indirect characteristics. Thus, for ungulates, it may be useful to locate pastures, watering places and migration routes (trails). Regular observations should be carried out in such places, noting systematically the appearance of fresh tracks, the specific assortment, approximate number and direction of migration of the respective animals. Relative carnivore counts may be based on the amount of baits which they devour in various parts of the forest.

Sufficiently detailed information on the number and distribution of game may be obtained on request from sporting establishments, hunting reserves and local huntsmen.

Domestic animals. Considering the great importance of domestic animals as hosts of adult ticks, the respective data on the former's specific assortment and number should be gathered from collective and individual farms in each focus. Simultaneously, all pastures and cattle trails in the locality under survey are plotted on a map with information on the permanency and time of their use. For many foci, such matter constitutes the basic initial data for all subsequent work.

UTILISATION OF VERTEBRATE COUNT FINDINGS

Count data, first and foremost, provide an idea of the vertebrate fauna on the area under survey, as well as its specific composition and distribution among habitats With the use of special symbols, the data are plotted on a key map, clearly showing the level of vertebrate population for different habitats and the habitats with the densest populations It should be borne in mind that various counting techniques characterise the fauna in different ways, being mutually complementary, owing to which the completest data are obtained by combined use of various techniques

Quantitative counts serve to establish the regularities in the seasonal and yearly fluctuation of animal populations in different habitats This furnishes the basis for determining those habitats in the natural focus that possess more stable animal populations, among which the species of relatively high and stable number (the potential principal hosts) may be noted alongside with those whose populations are high but unstable (secondary hosts) For birds, it is also important to define the ecological associations of each given ground species In the aggregate, these findings present a preparatory stage necessary for evaluating the importance of different vertebrates as tick hosts

Finally, observations of animal mobility carried out during quantitative surveys, offer a means of determining *the migration range of the respective species*, as well as seasonal and annual changes in their use of the area under survey This information forms a basis for estimating the extent of contact between different species and ticks, and the potentialities of the latter's dissemination through the area The findings on animal population dynamics, migration and seasonal concentrations, should likewise *be plotted on a map with the use of special* symbols All the obtained general biological data offer a starting point for further special research in the foci of tick borne encephalitis

SPECIAL RESEARCH IN FOCI OF TICK-BORNE ENCEPHALITIS

The special studies described hereunder are purposed to establish the role of vertebrates in natural foci, and include parasitological and virological investigations Both these aspects of research are interrelated, the choice of techniques employed for both being largely determined by the findings of general biological surveys

Parasitological investigation at its first stage ascertains the importance of individual species in the maintenance of ticks The basic techniques employed are collection of ticks by combing the animals, and estimation of the latter's importance as hosts

Most generally employed are data on relative tick-carrying importance which are obtained by computing the mean number of ticks per investigated or infested animal, the resultant indices being called, respectively, the abundance factor and intensive infestment rate, or by calculating the percentage of infested animals (extensive infestment rate) without regard to the size of the population of different species More workable data are obtained from analysis of information gathered at vertebrate population counts

In the case of relative population counts, use is made of information on animal infestment per counting unit, i e, the number of ticks collected per 100 trap-days or 10 pit days (for small mammals), per 1 kilometre of route (for birds, etc) The said factor, i e, infestment per counting unit, incorporates all the enumerated indices, with reference, however, to the relative animal and bird population, and may be used for comparison of infestment data for individual seasons, years and habitats However, since comparison between data per counting unit collected by different techniques, must needs be highly relative, only the figures obtained by one single method are practically comparable This shortcoming is non-existent in the case of estimates of actual tick-carrying importance, which are founded on absolute vertebrate counts and overall infestment assays, and are usually expressed in terms of the number of ticks per unit of area In the latter instance, despite distinctions of counting techniques, the materials obtained for different groups of vertebrates are mutually comparable

The precisest data on tick-carrying importance are obtained by regular combing of marked animals trapped on test grounds, which not only permits calculation of animal infestment per unit of area, but provides absolute data on the number of ticks maintained during the season Unfortunately, up to the present this method is applicable only for rodents An essential common drawback of absolute techniques is their laboriousness, and hence their restricted applicability on areas of any appreciable size, and total impracticability for certain groups, e g, *Insectivora* Issuing from investigations of vertebrates carried out for each stage of tick metamorphosis, the principal, intermediate and secondary hosts are established

Apart from determining the importance of separate vertebrates as tick hosts, the results of counts are used for solving certain problems of parasitological character Regular collection of material during counts permits the following to be clarified for the area under survey

(1) distribution through habitats and relative population of different tick stages,

(2) seasonal, or (in perennial surveys) annual regularities of parasitising on different species of animals, and, in particular, substitution of hosts from among the available host assortment, depending on the level of the latter's population,

(3) regularities in fluctuations of tick population associated with changes in the population of the host species,

(4) distribution of sectors of the area which possess permanent tick populations

The findings obtained are charted, using special symbols to outline tick foci for which detailed biotopic and faunistic descriptions are given together with square measurements of their area The location and description of areas harbouring permanent tick foci presents an important preliminary for the solution of the cardinal problem of detecting permanent foci of tick borne encephalitis on the basis of virological studies

Virological investigations are purposed to establish the importance of vertebrates in virus circulation in the natural foci as a whole, and their significance in maintaining the virus in individual sectors of the latter which are called elementary foci

The importance of vertebrates in the circulation of the pathogen through natural foci is established by testing their cerebrum and blood for the presence of the virus (detection of spontaneous infection) Besides direct assays of animal infection in a focus, there are a number of indirect methods, such as the establishment of complement fixing and virus neutralising antibodies in animal blood and their relative content in different species from different parts of a focus In small mammals and birds such tests may be carried out on liver and spleen extracts instead of blood

The virus carrying importance of animals is also established experimentally, by infecting different species of tick hosts in order to determine their susceptibility and the time and intensity of virus circulation in their blood (the degree of virusemia) The latter in donor animals determines the ability of ticks to receive the virus and maintain it through subsequent metamorphosis

In accordance with the cited techniques, virological investigations are conducted along three lines (1) assessment of spontaneous infection (2) serological studies (determination of animal immunity levels) (3) experimental tests of vertebrate susceptibility to the virus of tick-borne encephalitis Virological work on all these aspects is linked up with zooparasitological findings, particularly tick population and animal infestment data The stated principles should be borne in mind during the collection of material for investigations The most valuable results are obtained when collecting virological materials during vertebrate population counts

In estimating spontaneous infection and immunity levels, the most important subjects of observation are as follows (1) percentage of infected and immune individuals in different species and in different age groups of the same species in relation to their importance as tick hosts, (2) degree of infection and immunisation for individual species in certain habitats distinguished by high populations, (3) regularities of yearly and seasonal variations in infection and immunity of animals for different habitats, depending on the level of population of different species and tick infestment rate

Of special importance are virological investigations on the most widespread animals in the years when their population levels differ This work serves to elucidate the role of vertebrates in disseminating the virus through the area in different years and helps to establish the animal species responsible for changes in the epidemiological situation in natural foci, which is essential for epidemiological prognosis Definitive evaluation of the importance of individual vertebrate species in the circulation of the pathogen of tick borne encephalitis, is possible only after conducting susceptibility tests For this purpose the virus is introduced into the most important samples of animals and birds obtained from epidemiologically different habitats These tests are necessary to ascertain (1) the degree of susceptibility of various species, (2) the degree of virusemia in different species and the potentialities of tick infection from the latter, (3) transmission of immunity to the young in different groups of animals and birds

These findings will provide the material for definitive determination of principal and secondary pathogen carriers among the vertebrates in the natural foci All the virological data are plotted on a map providing a vivid picture of the distribution of elementary foci through the area and presenting the means of determining their size

The earliest measures to be adopted against ticks in our country were scientifically grounded and elaborated by Academician Y N Pavlovsky (1924 1937) in regard to a number of tick transmitted diseases The first recommendations for anti tick prophylaxis in encephalitic foci were likewise scientifically grounded by Y N Pavlovsky (1940) and his disciples G S Pervomaisky (1941) et al Individual prophylactic measures found wide application in tick borne encephalitis foci of the Far East from the very first years pending the discovery of the disease and have retained their importance ever since All subsequent work on the control of tick borne encephalitis has confirmed their leading significance

Anti tick prophylaxis is effected by the use of protective means preventing the attachment of ticks to the human body and various means of eliminating ticks over the area

Individual Protective Measures

Tucking in one s clothes The surest and simplest means of individual protection against ticks is to carefully tuck in one s clothes at all joints Shirts or blouses are tucked into tightly belted trousers Sleeve cuffs should fit as close as possible but are best tied with tape or elastic bands Trouser cuffs are tucked into tight socks or puttees the suit or coat collar should closely fit the neck

Tick proof clothing During field work in areas abounding in ticks it is advisable to wear special clothing whose cut prevents the penetration of ticks to the body e g a loose gym shirt and trousers made of strong stuff and if possible waterproof The material should be sufficiently porous to permit free evaporation of sweat

The khaki-coloured stuff should be smooth so as to hinder attachment of ticks The shirt too is made double for protection against mosquitoes and greater durability

The sleeves are long, wide and loose-fitting, except for the sleeve-ends, which should be tied with tape or provided with elastic bands. The same applies to cuffs, if present, as well as collars: best of all if the shirt is supplied with a zip-fastener. The trousers are made of stronger material and wider than usual. The waist is threaded with an elastic band or string, the free ends being tied up. The trousers should have double knee-caps and large surface pockets.

With clothes well tucked in, the only parts left exposed are the face, neck and occiput. These may be protected with a bandana of light, fine-meshed muslin.

The best foot-gear are boots, which should tightly fit the shanks to prevent penetration of ticks.

Self- and mutual inspection are obligatory with any kind of clothes. Observations have shown that inspections should be made twice a day (G. S. Pervomaisky's experiments in the Primorye territory).

Repellents. To prevent ticks from getting under the collar, it is good to tie a Pavlovsky net round the neck in the manner of a scarf.

Social Protective Measures

Choice of camp site and arrangement of camps. A camp should be set up on treeless or sparsely timbered ground. The chosen site should be dry and open. Its area should be cleared of all deadfall, stumps and scrub. The camp should be located far enough from roads, trails, gullies, ditches, cattle routes, watering places and pastures, which usually abound in ticks. The grass around the camp is regularly mowed and removed as far as possible. A few days before the workers' arrival, the camp area and adjacent forest within a radius of 25 to 30 metres should be thoroughly treated with hexachlorane or DDT using 30 to 50 kg of 10 per cent powder per hectare. All wood-felling sites should be known beforehand and prior to the beginning of work may also be treated with acaricides No garbage dumps are permissible near the camp, being a lure for rodents which may bring ticks

Special precautions should be taken in laying roads and pathways through forests Roads should be not less than 2 5 and pathways 1 or 1 5 metres wide The marginal area along roads and pathways should be freed of vegetation or treated with acaricides within 2 to 3 metres from the road

When choosing a site for short-term camping (not more than 10 days) the same rules are observed, omitting only large scale operations involving clearance and acaricide treatment It will suffice to thoroughly clear the ground before pitching tents, surrounding it with a tick-proof barrier of mowed or acaricide treated vegetation within a radius of not less than 3 to 5 metres Sometimes both methods may be combined

The same requirements are set to the choice of forest areas for children's rest camps and health centres (preliminary clearing and acaricide treatment are mandatory)

Tick control In controlling ticks, wide use is made of hexachlorane or DDT preparations sprayed from aircraft or helicopters over extensive forest areas worked by large numbers of lumbermen Acaricide expenditure amounts to 3 5 gr of 10 per cent mixture per sq m, or 30 to 50 kg per hectare (N I Gorchakovskaya, V A Nabokov et al)

Forest treatment should be carried out early in spring prior to foliation or even spring thaw Some authors advise spraying forest areas in autumn, before snow fall, in which case the preparation subsides on the litter and retains its qualities until springtime The ticks come into contact with the poison in spring, after hibernation, when first emerging on the litter surface In aircraft spraying, the acaricide evenly covers the litter, pathways, and roadside areas and may cause the death of up to 95 or 100 per cent of the ticks

Various species of *Ixodidae* may be pests of both domestic animals and man In veterinary practice, tick control measures have long been employed in all varieties, direct (spraying or bathing of cattle with arsenic solutions) and indirect (rational and regular changes of grazing ground) For epidemiologists, however, the control of ticks presents a comparatively new field, since in the U S S R their noxious effects on man were established only 25 years ago Owing to the fact that ticks are a "two way" pest, practical tick control measures in areas of various nature should be evolved in collaboration by epizootologists, epidemiologists and parasitologists, in the course of combined programmes for the development of the respective techniques, and in practical control campaigns [1]

Vaccination The population may be safeguarded against tick-borne encephalitis by mass vaccination, which particularly *refers to groups of people to be engaged in pro*longed work in forests during the period of maximum tick activity Vaccination should likewise be extended to the *families of timber workers inhabiting newly built forest* settlements Vaccination should be conducted from 1 5 to 2 months prior to the beginning of the tick season Sufficient vaccine should be available for use in *all* regions with natural foci of the disease

Therapy All persons contracting the disease require immediate hospitalisation

If the hospital is far off, the patients should be delivered by air ambulance

One of the chief therapeutic measures is the use of hyperimmune horse serum The latter should be freed of proteins to prevent serum disease Treatment may also be effected with serum from post-convalescents The use of this form of treatment, however, may often be limited by local possibilities

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[1] The use of raw goat milk causes a cerebral disease called two wave meningo-encephalitus (A A Smorodintsev S N Davidenkov) Omitting the problem of the specificity of this and other neuroinfections we must mention the new fact that the incidence of such afflictions is observed in people with abnormal changes of gastric juice whereas normal juice kills the virus This is a circumstance of major significance not in the least contradictory to the leading importance of ticks as reservoirs of the pathogen and vectors who infect the animals imparting pathogenic properties to their milk —Y *Pavlovsky*

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ASIAN TICK TYPHUS

Synonyms *North Asian tick typhus, tick borne rickettsiosis, Ixodo rickettsiosis asiatica, Siberian tick typhus*

Asian tick typhus is an acute infectious disease with *sudden onset, fever, primary lesions in the form of ulcers* in the place of tick bite, increased regional lymphatic nodes, *roseolous-papulous eruption, and bradicardia*

The disease was discovered in 1938 in the neighbourhood of Krasnoyarsk by an expedition of the All-Union Institute of Experimental Medicine, headed by Prof M K Krontovskaya During the expedition the rickettsial origin of the disease was established, O S Korshunova *having isolated* rickettsial strains from patients' blood The pathogen was proved to be transmitted from animals to man as a result of suction by mature spontaneously infected pasture ticks *Dermacentor nuttalli* Olen (P L Soliterman and S P Piontkovskaya) Its natural reservoirs were likewise found among the principal hosts of larvae and nymphs of vector ticks *the long-tailed suslik, narrow skulled vole and grey* rat (M K Krontovskaya) Clinical and epidemiological investigation of the disease was also conducted (M D Shmatikov, M A Velik, 1939, M K Krontovskaya, 1943) Experimental proof was offered on the long-term maintenance of rickettsiae during the metamorphosis of the tick *D nuttalli*, and its transovarial transmission (S P Piont*kovskaya*, O S *Korshunova*, 1940)

The aforenamed Soviet workers clarified the etiological, clinical and epidemiological features of the newly discov ered rickettsial disease, and established the main aspects of the natural focality of this infection, which they called tick typhus The findings of the expedition as well as data on tick borne encephalitis and certain other diseases were used by Academician Y N Pavlovsky (1938) as a basis for the theory of natural focality which he was then starting to build up Subsequent investigations by various authors established the widespread occurrence of tick typhus foci in part of the Asian U S S R

The tick typhus pathogen isolated by O S Korshunova (1938) from patients' blood, was the first rickettsia pathogenic to man revealed in the Soviet Union The discovery was made with the use of guinea pigs, white rats and rabbits Guinea pigs infected intraperitoneally with 3 to 4 ml of blood from a patient in the first stage of the disease, developed experimental infection with the characteristic two wave temperature curve accompanied by the scrotal phenomenon Vaginal smears revealed polymorphous rickettsiae situated intra and extracellularly 0 2 to 0 5 ml of visceral suspension from the infected guinea pigs, injected into the tests of experimental rabbits, caused the development of orchites, while subcutaneous inoculation led to inflammation and infiltration followed by necrosis Injections into the anterior chamber of the eye developed specific iridocyclitis with rickettsiae occurring in the cells of Descemet's membrane (P L Soliterman, O S Korshunova, 1938, 1939) From 8 to 10 days later, the sera of infected rabbits showed a positive Weil-Felix reaction with proteus OX_{19}, which was less intensive with OX_2, however gradually becoming more intensive, and seldom being positive in low titres with OXK

White mice inoculated intraperitoneally with a visceral suspension from infected guinea pigs, developed a symptomless infection (O S Korshunova, 1943)

E P Savitskaya (1944) in experiments on monkeys (*Macacus rhesus*) confirmed the specificity of the earlier isolated pathogen In monkeys the disease assumed a febrile course, continuing from 5 to 7 days after 3 to 4 days incubation, the Weil Felix test being positive I V Davidovsky (1940) detected rickettsiae in strips of biopsied skin *from the sites of tick bite*

After detailed cytomorphologic investigations, P F. Zdrodovsky and E M Golinevich (1949) gave the microbe the name of *Dermacentroxenus sibiricus*, after the place of its discovery, substituting the term tick typhus for North Asian rickettsiosis However, at the *present level of knowledge*, considering the distribution of the disease, it seems more suitable to call the disease proper Asian tick typhus, and its agent *R asiatica*, all the more so as the reservoirs of the disease in foci were found to be not only *Ixodidae*, but also *Gamasidae*, *Trombiculinae* and fleas, whose importance in transmitting the pathogen to man must not be ruled out (O S Korshunova, S P Piontkovskaya, 1957, M S Shaiman, 1957, M I Shapiro, 1957)

Morphological studies of the pathogen on smears and prints obtained chiefly from the scrotal membranes of infected guinea pigs, and stained after Romanowsky Giemsa, Macchiavello and Castaneda, showed its marked polymorphism single and coupled cocci and bipolar bacilli (occasionally, the cocci may form chains)

The stated picture is especially demonstrative in dark-field examination The microbes are arranged intra- and extracellularly A rickettsial suspension purified by fractional centrifugation in saline and stained with carbolfuchsine, resembles bacteria

R sibiricus is filtrable through macroporous Berkfeld filters (T A Skorin and O S Korshunova, 1941) Like other rickettsiae, they are rather resistant to low temperature and can stay alive at 30° C below zero for 3 years The pathogen is quickly inactivated by air, but survives for long periods when dried, especially by the lyophile procedure Drying is an effective method of preserving rickettsiae, much used for the purpose *in laboratory practice* Observations by S P Piontkovskaya and O S Korshunova

(1953) proved the possibility of survival of *R sibiricus* in experimentally or spontaneously infected ticks *Dermacentor marginatus* Sulz during five years with subsequent transmission to the fourth generation

R sibiricus survive in the viscera of white rats for up to 140 days, which, possibly, is not the limit (O S Korshunova, 1943)

The immunological and serological features of the pathogen are of great theoretical and practical importance for differentiating individual groups of rickettsiae and also for prophylaxis Investigations by O S Korshunova (1939) clarified the immunological peculiarities of rickettsiae, which allowed to conclude that immunologically, the most complex among them are *R prowazeki*, followed by *R sibiricus* and *R conori* Experimental cross-immunity data are summarised in Table 1

Table 1

Experiments in Cross Immunity

Primary inoculation	Repeated inoculation	Number of guinea pigs		Occurrence of immunity
		Total	Sensitive	
Tick typhus (Krasnoyarsk strain B)	Tick typhus (Krasnoyarsk strain B)	16	0	Apparent
Ditto	Typhus (Otto s strain)	6	6	Absent
Ditto	Mediterranean fever (Crimean strain)	4	0	Apparent
Typhus (Otto s strain)	Tick typhus (Krasnoyarsk strain B)	7	3	Relative
Mediterranean fever (Crimean strain after A Y Alymov, 1939)	Ditto	3	3	Absent

The cited findings were confirmed in subsequent investigations Parker and Castaneda (1943) and Megaw (1952) note the marked degree of immunological affinity between typhus and the Rocky Mountain spotted fever group However, R *rickettsi* (the causative agent of Rocky Mountain spotted fever) does not fully immunise guinea pigs against infection with R *prowazeki* Animals infected with Mediterranean fever do not acquire immunity against R *rickettsi*

Giroud, Le Gac and Roger (1947) obtained a distinctly positive agglutination reaction for R *prowazeki* with sera from Mediterranean fever patients In 1953 the same authors reported on the high variability of a strain of R *rickettsi*, having even noted morphological changes in the rickettsiae which stopped reproducing in the cellular nuclei, i e, lost their distinctive specific properties During these experiments the French authors carried out nasal inoculations of white mice (Giroud, Le Gac, Roger, 1953) Stuart Harris (1955), in his latest handbook on viral and rickettsial diseases, states that the cross immunity reaction between R *prowazeki* and R *mooseri*, on the one hand, and R *rickettsi*, R *conori*, and R *akari*, on the other, have not been studied in detail Thus, serum from patients with Rocky Mountain spotted fever may react positively to antigens from R *prowazeki* and R *mooseri* in the complement fixation and neutralisation tests Apparently, R *rickettsi* possess a common antigen group with R *prowazeki* and R *mooseri*

The above data do not agree with those of P F Zdrodovsky and L M Golinevich (1954) who state the absence of serological affinity between the pathogens of tick borne rickettsioses (D *sibiricus*, D *conori*, D *murinus*) and the rickettsiae of the typhus group (R *prowazeki* and R *mooseri*), regarding serologic analysis as the basic method for differentiating rickettsiae

Differential diagnosis The diagnostic methods used in all rickettsial diseases are the following animal inoculation, Weil Felix's test with proteus OX_{19}, OX_2 and OXK complement fixation with antigens from R *sibiricus*, R co *nori, R murinus, R prowazeki, R mooseri* and *R burneti* with obligatory analysis of group reactions In some cases it is necessary to study the antibody growth dynamics In animal inoculation, the blood of a suspected patient is injected intraperitoneally to male guinea pigs in volumes of 3 to 4 ml With the onset of disease in the animals, fresh series should be carried out, inoculating new guinea pigs with blood and visceral suspension from a diseased guinea pig in the second or third day of fever Convalescent guinea pigs are tested for cross immunity, taking their sera on the 24th or 25th day of the disease for complement fixing with standard antigens from the aforenamed rickettsiae It is also advisable to inoculate six- or seven day old chicken embryos, injecting the sera into the yolk sac, and in appropriate laboratory conditions, to be used for infecting white mice (nasally)

Therapy At present, the problem of therapy in Asian tick typhus, as all other rickettsial diseases, is solved in essence by the use of the antibiotics—aureomycin, chloromycetin and terramycin, if available, whose efficacy has been asserted in the clinic and laboratory by Soviet and other authors The results of extensive experiments with aureomycin were published by Wong and Cox (1948), and Anigstein, Whitney and Beninson (1948), for chloromycetin—by Smadel and Jackson (1947), Smadel, Jackson and Cruise (1949) In 1950 *Smadel, Jackson, Ley and Rose* published their study on the efficacy of terramycin In the Soviet Union, N G Kekcheyeva (1954) successfully applied experimental aureomycin and biomycin treatment to vesicular rickettsiosis in white mice The efficacy of aureomycin and biomycin was equal in all experiments

Clinical observations by many authors fully confirmed the results of laboratory experiments All the aforenamed antibiotics are in effect rickettsiostatic and not rickettsicidal, i e, inhibit the growth of rickettsiae and enhance the development of protective mechanisms The dosing is as follows 2 or 3 grams of the preparation daily, per os, until a fall in temperature, and 24 to 48 hours afterwards For chloromycetin Smadel (1951) recommends the use of a 3 gram initial shock dose It is advisable to wash down each dose of antibiotic with a glass of some kind of liquid, preferably milk Harrell (1949), summarising the therapeutic means against Rocky Mountain spotted fever, suggests applying 50 mg/kg of aureomycin and terramycin per os every 3 hours daily, as well as cortisone in combination with chloromycetin

Distribution Incidence of tick typhus has been established in Asiatic U S S R The range of tick typhus extends into the depth of the Asian continent, including its central part. Recently, *R sibiricus* has been revealed in ixodid ticks in the Armenian S S R (M Kotsinyan, 1959)

In Eastern U S S R natural foci of the disease are spread through the steppe and forest steppe, and are known in the superestuary areas of rivers belonging to the systems of the Amur, Yenisei and Ob The range of the pathogen extends to other zones as well *R sibiricus* has been found in ixodid ticks (Table 2) inhabiting southern semi deserts (basin of lake Zaisan, Kazakhstan), desert steppe and semi fixed sands (Ubsunur basin, Touva) Active and potential foci are in most cases associated with dry climate (sediments not more than 300 mm annually)

Close proximity to mountain ranges (Altai, Salair, Tien Shan, Sayan, Tannul Ola, etc) or location in interalpine (Zaisan, and Touva) and alpine (Usinsk, Ubsunur) depressions determine the common features of relief (mountains hills ridges) in many natural foci Among the known components of natural foci widespread in various regions in Eastern U S S R, we may name ixodid ticks of the genus *Dermacentor* (4 species), *Haemaphysalis* (2 species) and the wild animals serving as their hosts In many foci *Gamasidae* have been proved to take part in the circulation of *R sibiricus* among rodents (Eastern Kazakhstan Novosibirsk region, South Primorye) as well as chiggermites (Eastern Kazakhstan) and fleas (Khakassian region)

Table 2

Tick and Flea Species Known as Reservoirs of Rickettsiae

Reservoir species	Place of discovery	Authors responsible for establishment	
		Spontaneous infection	Transovarial and trans stage transmission
D nuttalli	Krasnoyarsk territory (near Krasnoyarsk and Kansk)	P I Soliterman S P Piontkovskaya, 1938	S P Piontkovskaya O S Korshunova 1941
D nuttalli	Near Krasnoyarsk and village of Novosyolovo	S P Piontkovskaya E V Arkhina O S Korshunova 1939	The same
D nuttalli	Khakassian region (Ust Abakan Bograd and Usinsk districts)	O S Korshunova S P Piontkovskaya 1956	The same
D nuttalli	Chita region	F D Petrovev 1946	—
D silvarum *Haemaphysalis concinna* Koch	Khabarovsk territory	L P Savitskaya 1943	L P Savitskaya 1943
D silvarum	Kemerovo region	D F Pletsity 1947	—
D silvarum *D pictus*	Novosibirsk region (Toguchinsk district)	M S Shaiman 1957	M S Shaiman 1957
D marginatus *D pictus*	Altai territory	S M Kulagin N I Alfeyev O S Korshunova 1947	S P Piontkovskaya O S Korshunova 1953
D marginatus	East Kazakhstan (Shemonaikha and Zaisan districts)	O S Korshunova S P Piontkovskaya 1957	S P Piontkovskaya O S Korshunova 1957
D marginatus *H punctata*	Alma Ata region	D S Arkhangelsky 1955	—
D marginatus *H punctata*	Kirghizia (Issyl Kul region)	M K Krontovskaya	M K Krontovskaya
		L P Savitskaya 1946 G A Kvitnitskaya 1947	L P Savitskaya 1946
H concinna	Southern Primoryo (Barabash)	Z M Zhmayeva 1950	Z M Zhmayeva 1950

(Continued)

Reservoir species	Place of discovery	Authors responsible for establishment	
		Spontaneous infection	Transovarial and transphase transmission
H concinna	Southern Primorye (island)	G P Somov M I Shapiro A A Petrov 1957	—
Hirstionyssus myospalacis sp n Zemsk et Piontk (from Altai zokor)	Eastern Kazakhstan (Shemonaikha district)	O S Korshunova S P Piontkovskaya 1957	—
H isabellinus Oud *Haemolaelaps glasgowi*	Island in South Primorye	G P Somov M I Shapiro A A Petrov 1957	—
Nothrholaspis sp (from common hamster)	East Kazakhstan (Zaisan region)	O S Korshunova S P Piontkovskaya 1957	—
Gamasidae (from burrows of narrow skulled vole)	Novosibirsk region (Toguchinsk district)	M S Shaiman 1957	—
Schoengastia rotundata	East Kazakhstan (Shemonaikha district)	O S Korshunova S P Piontkovskaya 1957	—
Neopsylla setosa (from long tailed suslik)	Khakassian region (Ust Abakan and Bograd districts)	O S Korshunova S P Piontkovskaya 1957	—
Ctenophthalmus arralis (from narrow skulled vole)[1]	Khakassian region (Ust Abakan and Bograd districts)	O S Korshunova S P Piontkovskaya 1959	

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[1] *Note* From suspensions chiefly including these flea species as well as some others

Ixodidae not only transmit the infective agent to animals and man, but present its stable reservoirs There is evidence likewise of the stage-to-stage and transovarial transmission of *R sibiricus* by ticks, and the maintenance of the agent through 4 successive generations of *D marginatus* (S P Piontkovskaya and O S Korshunova, 1959)

Cases are known when *R. sibiricus* was transmitted by spontaneously and artificially infected *D. nuttalli* during interrupted (with intervals up to 1.5 months) and uninterrupted blood meals on guinea pigs (S. P. Piontkovskaya; O. S. Korshunova, 1947, 1959). During their life cycle the ixodid vectors of tick typhus change three hosts. The *mature forms, larvae and nymphs take a single blood meal* apiece, each on a fresh host, each blood meal continuing for several days. In laboratory conditions, the development of ticks g. *Dermacentor* (from the detachment of the engorged female to nymph-moult) occupies approximately 2 to 2.5 months (*D. nuttalli*—S. P. Piontkovskaya, 1941; N. D. Yemelyanova, 1956; *D. pictus*—N. G. Olsufyev, 1953; *D. silvarum*—A. I. Shpringoltz-Schmidt et coll., 1935). Ticks of the genus *Haemaphysalis* have a longer life cycle, continuing from 3 (*H. punctata*—M. V. Pospelova-Shtrom and S. P. Piontkovskaya, 1949) to 5 and more months (*H. concinna*—B. I. Pomerantsev, 1950).

In nature, the life cycle of a tick population may increase depending on climatic conditions, number and assortment of hosts, periods of encounter with the latter (in each of the metacyclic stages), and famine resistance. Owing to the marked seasonal character of preying in the larvae and nymphs of *Dermacentor*, and the shortness, in laboratory conditions, of their life span as compared with other species, there is all reason to believe that the development of a single generation in this genus occupies one year (G. V. Serdyukova, 1950; N. D. Yemelyanova, 1959). For *H. concinna*, in nature this period is extended to 2 years (B. I. Pomerantsev, 1950). Since in nature, ticks are capable of prolonged starvation (*D. pictus*—3 to 4 years, N. G. Olsufyev, 1953; *D. marginatus* 2 years—E. I. Pokrovskaya, 1953), *with the lack of hosts, the imago forms are* liable to repeated hibernation. Owing to that, the life cycle of part of a generation may continue for 2 years and more.

Species of *Ixodidae* including vectors of tick typhus, are spread through different zones of the U.S.S.R. (Table 3).

The range of *D. nuttalli* in the U.S.S.R. has a northern boundary. Phylogenetically, this species is associated with the Central Asiatic species *D. birulai* (B. I. Pomerantsev, 1950). Its range is known to extend through north-western (N. O. Olenev, 1929; S. A. Svirskaya, 1941) and north-eastern Mongolia (M. N. Baidin, 1944), eastern Tibet and northern Tien-Shan (B. I. Pomerantsev, 1950). In the U.S.S.R. *D. nuttalli* occurs in the steppe along the mountain river Chu, Gorno-Altai region (P. V. Semyonov, 1954) and at 1,300 metres above sea level in the Ubsunur basin, Touva (S. P. Piontkovskaya, 1959). The range of *D. pictus* generally coincides with the mixed and deciduous forest zones (B. I. Pomerantseva, 1950) where it abounds in meadowlands, among scrub and on timber-felling sites (southern part of Moscow region—N. G. Olsufyev, 1953). This species is widespread in the forest-steppe of the Omsk (A. F. Fedyushin, 1949) and Novosibirsk (G. K. Lonsinger, 1957) regions.

The vectors' preying seasons are given in Table 3. Information on the phenology, habitat distribution and assortment of vector hosts is available for the Krasnoyarsk territory *(D. nuttalli*—S. P. Piontkovskaya, 1941, 1951), Touva basin *(D. nuttalli*—S. P. Piontkovskaya and N. K. Mishchenko, 1959), Altai territory *(D. marginatus, D. pictus*—N. I. Alfeyev, 1953), Novosibirsk region *(D. pictus, D. silvarum*—G. K. Lonsinger, 1957), Southern Primorye *(H. concinna*—Z. M. Zhmayeva, 1946). The preying season of *D. nutalli* in the neighbourhood of Krasnoyarsk is given below, the respective data for the Touva basin being the same (S.P. Piontkovskaya, 1958).

According to available information, the following ticks may spend the winter attached to cattle, though without sucking the latter's blood: *D. silvarum*—Khabarovsk territory (A. F. Kasyanov, 1947); Primorye territory—(A. I. Shpringoltz-Schmidt, 1935); *D. marginatus, D. pictus* (Omsk region—G. I. Getta, 1954); *D nuttalli*—Krasnoyarsk territory (S. P. Piontkovskaya, 1951).

Table 3

General Data on Ixodid Vectors of Tick Typhus
(after B I Pomerantsev G V Serdyukova et al)

Species	Distribution		Principal hosts		Active season		Tick stage found attached to man
	Geographic	Topographic	Imago	Larvae nymphs	Imago	Larvae nymphs	
Haemaphysalis punctata	South of European U S S R Caucasus Central Asia Kazakhstan Byelorussian S S R (I M Arzamasov 1957) Western Europe North Africa Asia Minor Iran Balkans Italy Spain Mediterranean islands Hungary	Alpine forests and steppes steppe desert semi desert	Large mammals cattle rodents birds	Birds small mammals seldom cattle sheep and goats reptiles	Spring autumn, at south of range—the year round	From spring to autumn	Mature
H concinna	Territories of Khabarovsk and Primorye Western Sayans Byelorussian S S R (I M Arzamasov 1957) Crimea Ciscaucasia Transcaucasia Uzbek S S R Kirghiz S S R Eastern Kazakhstan	Deciduous and mixed forests (of the type met in Colchis Amur Ussuri area or the desert floodland type) Drying tussocky swamps (Z M Zhmayeva	Large wild mammals cattle (M V Pospelova Shtrom 1936)	Small and large mammals birds seldom reptiles cattle goats sheep	From spring to autumn (peak in June)	From spring to autumn	Mature forms and nymphs

(*Continued*)

Species	Distribution		Principal hosts		Active season		Tick stage found attached to man
	Geographic	Topographic	Imago	Larvae, nymphs	Imago	Larvae, nymphs	
	Western Europe Iran	1941), birch-aspen groves (M S Davydova, 1957) Alpine taiga (P V Semyonov, 1954)					
Dermacentor pictus	From western boundaries of the U S S R to Kansk, Crimea, Caucasus Transcaucasia, Kirghizia, Kazakhstan, Western Altai, Britain, Germany, Poland	Mixed and deciduous forests	Large wild mammals, cattle, hedgehogs, hares	The same	From early spring to the beginning of June, from end of August At south of range, seasonal boundaries vary	From spring to autumn	Mature
D marginatus	South of European U S S R, Transcaucasia, Central Asia, Kazakhstan, south and east of Western Siberia, occurs in more northerly regions (Voronezh, Tambov, Western Cisuralia, Byelorussian S S R). Southern Europe	Level and alpine steppes, forest steppe alpine forests, southern semidesert (S P. Piontkovskaya 1957)	Large wild mammals, cattle	Small mammals	From early spring to mid June, from mid-August to October. In south and north of range dates vary	Summer months	Mature

D silvarum	Primorye territory, Cisamuria, Eastern Transbaikalia Buryatia, Kemerovo region Altai territory (P V Semyonov 1954)	Forest steppe, taiga under cultivation	Large wild mammals, cattle foxes, hares	Small mammals	From early spring to October (south of Primorye territory)	Summer months	Mature forms and nymphs
D nuttalli	Chita and Irkutsk regions Krasnoyarsk territory, Buryatia (S N Machulsky and G I Getta, 1954), Tuva autonomous region (G I Getta 1957) south east of Gorno Altai region (P V Semyonov, 1954)	Cisalpine and alpine steppe, forest steppe, desert steppe semi fixed sands (S P Piontkovskaya 1957 1959)	Cattle large wild mammals	The same	The same	The same	The same

The post hibernant activity of mature ticks *Dermacentor* is of marked seasonal character The ticks take blood meals on animals *en masse* and attack man from early spring to the beginning of June With the advent of heat, the ticks sit hungry in their shelters, and then, from the middle of August (or somewhat earlier or later, depending on local climate) again issue forth to suck blood In the Touva basin (600 metres above sea level) *D nuttalli* has been observed to attack man in July (S P Piontkovskava and N K Mishchenko, 1959) The ticks usually climb to the tops of dry undergrowth and there lie in ambush in typical poses, attaching themselves, on occasion, to passing cattle or human clothes Mature ticks of the genus *Haemaphysalis* usually repose on live grass as well, the active season of *H punctata* and *H concinna* extending from spring to the midsummer months The larvae and nymphs of *Dermacentor* parasitise during summer, while in August the engorged nymphs moult into imago The larvae and nymphs of *H punctata* and *H concinna* feed from spring to autumn

Apart from vector ticks, the natural reservoirs of *R sibiricus* are known to be *Muridae* and other *Rodentia* serving as tick hosts (Table 4) The periods through which they maintain the pathogen in nature have not been established O S Korshunova, S P Piontkovskaya, N A Nikitina (1959) reported the maintenance of *R sibiricus* by mature post hibernant steppe lemmings, long tailed susliks and narrow skulled voles in natural foci of the Khakassian region The animals were examined in May, before larvae of *D nuttalli* had begun parasitising them

Ixodid ticks (among them vectors of tick borne typhus) parasitise on a broad range of hosts In cultivated, especially cattle breeding localities, cattle are the main source of food for mature ticks of the genera *Dermacentor* and *Haemaphysalis* The younger stages usually feed on small mammals *H punctata* and *H concinna* may also parasitise birds

Table 4

Animal Hosts of Vector Ticks—Natural Reservoirs of R sibiricus

Species	Natural foci of tick borne typhus	Method of establishing natural infection: Isolation of pathogen strain	Method of establishing natural infection: Complement fixation (with R sibiricus antigen)	Author
Narrow skulled vole (*Stenocranius gregalis* Pall)	Near Krasnoyarsk	+	—	M K Krontovskaya 1938, S P Piontkovskaya A P Bolayeva O S Korshunova, 1939
Ditto	Khakassian region (Ust Abakan district)	+	—	O S Korshunova, N A Nikitina, 1956
Steppe lemming (*Lagurus lagurus* Pall)	Khakassian region (Bograd district)	+	—	O S Korshunova, N A Nikitina, 1956
Domestic mouse (*Mus musculus* L)	Khakassian region (Ust Abakan district)	+	—	O S Korshunova, N A Nikitina, 1957
Brown hare (*Lepus europaeus* Pall)	Khakassian region (Altai district)	+	—	O S Korshunova, N A Nikitina, 1957
Long tailed suslik (*Citellus undulatus* Pall)	Near Krasnoyarsk	+	—	M K Kr[illegible]tovskaja, [illegible] O S K[illegible]nova, E [illegible] [illegible]khina, [illegible]
Ditto	Khakassian region (Bograd and Ust-Abakan districts)	+	1 80	O S [illegible]nova [illegible] [illegible]kitina [illegible]
	Usabasin (Village of V Usinskoye)	—	1 80	[illegible]
Grey rat (*Rattus norvegicus* Berk)	Near Krasnoyarsk	+	—	[illegible]

(Continued)

Species	Natural foci of tick borne typhus	Method of establishing natural infection		Author
		Isolation of pathogen strain	Complement fixation (with R sibiricus antigen)	
Field mouse (*Apodemus agrarius* Pall)	Khabarovsk territory	+	—	E P Savitskaya 1939
Reed vole (*Microtus michnoi* Rotsch)	Khabarovsk territory	+	—	I P Savitskaya 1939
Far Eastern hamster (*Cricetulus barabensis* Pall)	Khabarovsk territory	+	—	L P Savitskaya 1939
Chipmunk (*Eutamias sibiricus* Laxm)	Khabarovsk territory	+	—	I P Savitskaya 1939
Rat (*Rattus norvegicus caraco*)	Khabarovsk territory	+	—	E P Savitskaya 1939
Red cheeked suslik (*Citellus erythrogenys* Brandt)	Novosibirsk region (Toguchinsk district)	+	—	M S Shaiman 1957
Common hamster (*Cricetus cricetus* L)	Novosibirsk region (Toguchinsk district)	+	—	M S Shaiman 1957
Narrow skulled vole (*Microtus gregalis* Pall)	Novosibirsk region (Toguchinsk district)	+	—	M S Shaiman 1957
Field mouse (*Apodemus agrarius* Pall)	Novosibirsk region (Toguchinsk district)	+	—	M S Shaiman 1957
Reed vole (*M michnoi*)	Island in Southern Primorye	+	1 160	G P Somov M I Shapiro A A Petrov 1957
Grey hamster (*Cricetulus migratorius* Pall)	Armenia	+	—	M I Kotsinyan 1959

Symbols + strain isolated —test not made

The distribution of ticks through individual areas is usually associated with the ecological features of the principal hosts Formidable tick foci usually spring up in the places of concentration of animals serving as blood donors to all the metacyclic stages, and in localities providing favourable macro- and microclimatic conditions for metamorphosis, hibernation, oviposition and subsequent development of eggs in the environment Since the vectors of tick borne typhus have to change three successive hosts in the course of development, the distribution of man-attacking post-hibernant ticks in nature depends on the local distribution of the principal hosts of the pre imago stages (nymphs) Thus, in tick borne typhus foci of the Krasnoyarsk territory and Touva basin, the most abundant collections of *D nuttalli* are made on wormwood and cereal grown areas inhabited by the long tailed suslik The incidence of this animal's burrows is a sure landmark for detecting *D nuttalli* in both virgin and cultivated localities In the latter, the survival habitats of the suslik and concentrations of *D nuttalli* occur on field margins, roadsides and hillsides (Kansk and Usa districts) Information is likewise obtainable on the local distribution of other vectors in tick borne typhus foci Thus, in one of the foci of Southern Primorye, *D silvarum* abounds among the scrub on hills and mountains (A A Preobrazhensky, 1957) In one of the foci of the Novosibirsk region this species is prevalent in birch groves, scrub grown forests and on newly-felled mixed forest areas (G K Lonsinger, 1957) In the Altai foothills, *D marginatus* and *D pictus* are prevalent in thickets of dry wormwood and copses of birch and aspen (N N Alfeyev, S M Kulagin, 1953) In Southern Kazakhstan, *D marginatus* is mostly prevalent in the foothills of the Transilian Ala-Tau (E N Bartoshevich, 1957) *H concinna* in one of the foci of Southern Primorye is typical for habitats occupied by drying tussocky swamps (Z M Zhmayeva, 1940)

Ixodid ticks, small wild mammals and cattle are the principal components of natural foci of this disease, some being vectors and reservoirs of the pathogen in nature, and others acting as donors for the said ectoparasites. Hence, anti-tick prophylaxis should include in its scope the entire combination of species participating in the maintenance of the pathogen in the given locality. At present there exists a variety of mechanical, chemical and agrotechnical measures advisable for reducing the population of ixodid vectors and their hosts. In drawing up control programmes, attention should be paid to concrete local conditions (natural and economic) in each given focus. On this aspect, however, there is comparatively little published information. For the Krasnoyarsk territory, V. A. Nikonov (1953) advised treating cattle with 10 per cent DDT powder to destroy *D. nuttalli* (70 gr per animal). S. P. Piontkovskaya (1951) reported the efficiency of burning dead growth in spring. The number of ticks in an area subjected to burning near Krasnoyarsk was many times less than in an unburned control (Fig. 10). F. I. Dzyubak and S. S. Deg-

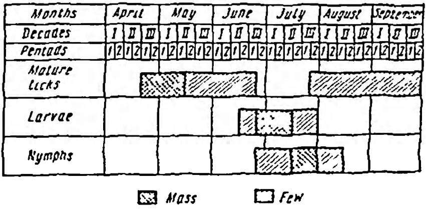

Fig 10 Feeding seasons of *Dermacentor nuttali* in the neighbourhood of Krasnoyarsk (S P. Piontkovskaya, 1939).

tyaryov (1956, Khabarovsk territory) advised eliminating *D. silvarum* by hand- or machine-spraying the respective area with hexachlorane (1 gr ADV per sq m of the area) The same authors proposed destroying *H concinna* in nature by use of 0 3 to 0 5 gr ADV per sq m In other regions, outside the range of tick typhus, *D pictus* was successfully destroyed by treating cattle, sheep and goats with DDT (50 gr of 10 per cent powder per head), the treatment being repeated 4 or 5 times during the season, with 7-day intervals (L N Pogodina, 1951)

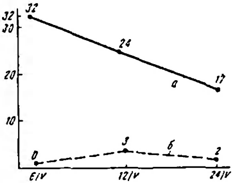

Fig 11 *Amount of adult ticks Dermacentor nuttalli* on 0 5 hectare area with (a) old and (b) burned grass (collected by hauler)

On cattle farms, it is advisable to plan and effect pasture turnover in accordance with the phenology of the vectors In foci harbouring *D nuttalli*, apart from the measures already mentioned, it is recommended (a) to burn the dry growth on steppeland pastures early each spring (Fig 11), doing it in the shortest time possible, and (b) to eliminate, in forest-steppe areas, all small sized thickets occurring on ploughland, regularly ploughing up and weeding all roadsides and spaces between fields

A prominent item in the general system of prophylaxis against tick typhus and other diseases with natural foci, is rodent control

The basic means of individual prophylaxis are regular inspections of the body and clothing for ticks (see chapter on tick-borne encephalitis)

In many of its aspects, the problem of tick typhus still awaits solution Besides elucidating its nosogeography (determining the northern and western boundaries of pathogen distribution), the structure of the respective natural foci (assortment and number of hosts of *R. sibiricus*, and the entire nomenclature of ectoparasites), the phenology and ecology of their principal components and the part played by each of the latter in maintaining the infection, it is important to clarify the daily routine and life span of mature vectors, and to determine the percentage of rickettsia-carriers in the population of post-hibernant ticks at springtime or pre-autumnal collection (for *Dermacentor*—late in August-September). Other points to be cleared are the length of the pathogen-carrying period for reservoirs in nature and the content of complement-fixing antibodies for *R. sibiricus* in the blood of cattle, as well as goats and sheep.

No less urgent is the development of more efficient methods for detecting and counting mature vectors and their pre-imago stages in nature, the elaboration of more perfect methods of eliminating the vectors in their habitats, and the invention of effective repellents for protecting people against ticks.

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COLLECTION OF IXODID TICKS AND OTHER ANIMAL ECTOPARASITES IN TICK TYPHUS FOCI[1]

A natural focus of disease may include numerous components (arthropods and mammals) intimately connected by various biotic interrelationships For some regions there is proof that *R sibiricus is circulated, among others, by Gamasidae, Trombiculinae* and fleas. Hence, investigations in such foci should embrace the entire assortment of animal ectoparasites (*in areas populated by H punctata and H concinna birds* should be taken into account as well) with the view of determining their species, number and importance in maintaining and transmitting the pathogen

Ixodid Ticks

The varied circumstances in which the tick vectors of the tick typhus pathogen may occur during their life cycle, e g, prolonged periods of residence in the fissures and on the surface of the soil (*for hibernation, oviposition and moulting*) on plants (preparing for attack) and during attachment to the host, which may occupy several days in each state of development, necessitate the use of different methods of collection from the terrain and bodies of wild and agricultural animals

Epidemiological surveys should cover the premises of human dwellings, adjoining yards and gardens all domestic animals including cattle maintained in cattle yards, stacks of hay and straw, fleeced wool, and all other objects liable to contact with patients. In the case of large mammals, such *surveys should include* inspection and palpation of the head chest, neck, back, and the abdominal and pos*terior surfaces of the groins* Domestic and field rodents (rats susliks, hamsters gerbils jerboas, etc.) are caught with snares and other rodents and *Insectivora*—with mouse traps Hay, straw and wool are picked over a sheet taking these materials in successive portions from stacks ricks etc

The typical biotopes of various species of *Dermacentor* and *Haemaphysalis* are areas of steppe forest steppe and occasionally the banks of rivers and lakes *visited by cattle* or *inhabited by rodents* and *Insectivora*, as well as ground nesting birds (hosts of *H punctata* and *H concinna*) The habitats where ticks concentrate are *located* by investigating various localities (with due regard for microtopographic details) on cultivated and uncultivated terrain natural grazing grounds and haymaking areas, hill sides, mountain flanks etc

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[1] This section is written by *S P Piontkovskaya*

In open steppe and forest steppe habitats ticks are collected (a) by hand from dead grass, counting the yield per unit of area (b) by means of a so called hauler (Nuttall s method) of rough cotton fabric or flannel of light, uniform colouring 100×45 or 120×60 cm in size, one of whose narrow hems is attached along a pole, the other being fitted with ribbons by which the surveyor trails it on his right-hand side over areas covered with low grass In forest steppe areas the same purpose is served by a flag of the aforesaid stuff 100x45 cm in size, fixed to a handle This device is drawn gently over scrub, tall grass, dry stalks or the grassy undergrowth in closed biotopes (the devices employed for tick collection are shown in Fig 6)

When investigating pasturelands for hungry larvae of *D marginatus*, the work may be done with a so called spindle representing a piece of flannel wrapped several times round a stick and secured by a string By a drill like motion, the device is slowly driven into bushes to about half their height

The larvae and nymphs of *H punctata* and *H concinna* are collected from small mammals and birds Susliks, hamsters, gerbils, gerboas, etc, are caught with traps left at dawn or sunset at the entrances to burrows Traps for susliks and hamsters should be examined and reset several times during the day, or, for nocturnal animals at night or at dawn *Muridae* are caught with mouse traps which are set at nightfall 25 50 100 in a row, at five metre intervals in different microhabitats of the area under survey, e g, clumps of grass or scrub, hollows, etc

The bait is made of finely chopped pieces of rye bread and carrot dressed with vegetable oil The traps are examined at daybreak Some animals, like the narrow-skulled vole, field mouse and steppe-lemming, are dug out of their burrows together with their nests on plots of sown grass or cereals Domestic mice are caught with mouse traps set at hole entrances inside and outside the premises Birds are shot Each animal, regardless of the method of collection is tied securely into a white cotton bag with a label showing the point, habitat, date and hour of collection, as well as the name of the collector The animals should be removed from traps as quickly as possible, since the fleas and *Gamasidae* will soon abandon the cooling carcass

In the laboratory, the animals are combed with a fine comb or tooth brush, inspecting their ears and going through the fur with a fine needle and tweezers After that the bag is turned inside out over an enamel trough whose sides are smeared with vaseline All the revealed ectoparasites are counted if possible, making special note of *Ixodidae* and *Gamasidae Trombiculinae* and fleas after which, by means of a needle dipped into alcohol, they are placed into separate entomological test tubes with 70° alcohol Records of ectoparasite counts are kept in a special notebook whose pages are ruled as follows

Animal	*Ixodidae*			*Gamasidae*	*Trombiculidae*	Fleas	Lice
	Imago	Larvae	Nymphs				

The table should contain entries on the total number of ectoparasites revealed at each combing (for animals) or inspection (for birds) At the end of a survey, all count results are entered in a journal with special columns for each group of ectoparasites The ectoparasites taken from each animal are placed into separate test tubes together with labels written in black ink showing register number and species of animal, point, habitat, date of collection and name of collector On doing that, the test tubes with 70° alcohol are stopped with cotton wool plugs dipped into alcohol (avoiding access of air bubbles) and placed into wide necked glass jars The bottom of each jar is lined with cotton wool, on which the test tubes are set upright in rows separating the latter with wads of cotton wool to avoid shaking The whole is covered with cotton wool, on which a second layer of test tubes is placed, and so on The jar is filled with 70° alcohol, tightly closed with a cork stopper, bound with gauze and sealed with paraffin. In this manner collections may be kept for years The jar is supplied with a label showing register number host, place and time of collection

Mature ticks are identified by means of a binocular lens or stereoscopic microscope, type MBS 1 A live hungry tick is examined by placing it between thin slides gently pressed together at the ends by two rubber bands

Before examination, alcohol treated ticks are dried with filter paper, and placed in the folds of a strip of corrugated paper or else in plasticine or cork The tick is examined from different angles under direct light

The systematics of juvenile forms of *Dermacentor* and *Rhipicephalus* have not been sufficiently studied Hence, on removal from hosts, the larvae and nymphs of these species are left alive to develop into mature forms feeding them on laboratory animals

Gamasidae, fleas and burrow inhabiting species of *Ixodidae* are collected by excavating the burrows of their hosts The content of burrows and small birds nests is placed into separate cotton bags together with labels bearing the usual data, and firmly tied During delivery to the laboratory, the bags should not be permitted to overheat, and, if immediate examination is impossible, should be kept in a dark, cool place The burrow substratum containing the ticks and insects, is examined by small portions in a bowl For convenience of work and completeness of collection, it is advisable to use thermoeclectors, which are available in several modifications A convenient device of this kind is a white tin funnel 25 cm in dia and 30 cm long with a close fitting lid and a neck 7 cm long and 8 cm in dia, which is tightly inserted into an iron can 7cm high. To the inner walls of the funnel is soldered a hoop securing a rare meshed iron net on to which the nest being examined is placed The can is filled with a water solution of glycerin The thermoeclector is set on a tripod in the sunshine, or else an electric lamp is installed over the lid of the funnel The heat forces the inhabitants of the nest to crawl down into the can, where they are extracted from the glycerin by a pair of tweezers, a needle, or fine brush, dried on filter-paper and, depending on the purpose of the work, placed into test tubes with 70° alcohol or sawdust

The results of parasitologic surveys of different areas, animals and premises are registered in blank form No 1

Maintenance and feeding of Ixodid ticks The maintenance of ixodid ticks, including underfed specimens collected from their natural habitats or animals for observation of development, is effected in the laboratory by feeding on guinea pigs, white mice, rabbits, etc Feeding on guinea pigs may be used for isolating rickettsial strains Ticks removed from wild animals may also be tested for spontaneous infection by the suspension technique Ticks collected in field conditions and from large agricultural animals are placed into bacteriological or flat-bottomed entomological test tubes which are closed with tight-fitting stoppers wrapped in gauze The equipment required for the work includes blunt tipped tweezers, a soft, thin camel hair paint-brush, and a notebook and soft pencil, all of which are placed into a field bag together with a supply of test tubes wrapped in paper and placed into the bag with their corks upward In the laboratory, ticks are maintained in test-tubes with moist softwood sawdust

The latter are prepared as follows A test tube is half filled with a loose mass of large grained sawdust, preferably sterilised, which is covered at the top with a filter-paper disc cut to the diameter of the test tube The space above the sawdust is occupied by a corrugated strip of the same paper Moistening is effected by means of a Pasteur pipette, wetting only the lower layer of sawdust For ticks populating dry open habitats (e g, *D nuttalli*) it will suffice to moisten only one third of the volume of the sawdust *D pictus*, *D marginatus, Rhipicephalus* and *Haemaphysalis* require higher humidity, i e, the lower half of the sawdust should be moistened For the moisture loving species of *Ixodidae* and certain species of *Haemaphysalis*, almost the entire mass of sawdust requires moistening Different tick stages require different humidity, which is determined by observation Excess moisture is fatal for the ticks, the free part of the test-tube and the paper strip should be dry If condensed vapour or drops of water from the pipette occur in the test tube, the latter should be carefully wiped from the inside with cotton wool held in a pair of tweezers When maintaining mature ticks engorged females and nymphs, an overmoistened test tube is closed for several days with a piece of gauze instead of cotton wool The need for re-moistening the sawdust depends on the general humidity of the room where the ticks are maintained The test tubes are regularly examined (not less than once a week)

Test tubes containing ticks are kept upright in test-tube holders, boxes, etc., shading them with black paper For prolonged maintenance of hungry ticks, it is advisable to keep them at temperatures from 2 to 18° C To accelerate the development of replete ticks (during moulting or oviposition) the test tubes should be kept at temperatures from 24 to

Record Form No 1

Number of Ticks and Fleas

No of collection	Date	Point	Habitat	Collection technique	Substratum and number of nests burrows, etc Details of habitat	Species	Animal						Number of ectoparasites collected from each animal						
							Males		Females		Young		Ixodidae				Gamasidae	Trombiculidae	Fleas
							Live	Dead	Live	Dead	Live	Dead	Females	Males	Larvae	Nymphs			

28° C in special hotbeds or in boxes fitted with internal electric conduit, or else, in thermostats or polythermostats with thermoregulation

When studying the metamorphosis and life cycle of ticks or testing them for spontaneous infection, the ticks are fed on laboratory animals The experimental animals of choice are rabbits, guinea pigs, and white mice Rabbits and guinea pigs are convenient for feeding all stages of three-host ticks Rabbits are likewise advisable for feeding large numbers of ticks in any metacyclic stage, as well as larvae of two host species which moult into nymphs without detachment from the host, a process which may occupy two weeks or more One animal is used for simultaneous feeding of all ticks of a single definite species (preferably, also, in the same stage of metamorphosis) collected from the same host and in the same place

Tick feeding requires the following equipment operating board and bandages for immobilising the animal's extremities, tin number tag, razor blade, curved scissors, tweezers, cloth sleeve, glue, cardboard, film or tin collar, needle and thread, piece of wire, soft brush, cage on tray, and journal for registering the data obtained The sleeve is used to prevent the ticks' escape and is made of cotton, with a square or circular cross-section, cut to the size of the area to be shaved on the animal's back. A strip of the same material 5 10 cm long is sewed to the base of the sleeve, producing an object shaped like a top hat. The base strip is smeared with glue—condensed collodium or a preparation of small bits of emulsion free exposed film dissolved in acetone The collar is made circular in shape, with a hole in the middle to fit the animal s neck, the rim at one point being cut through The collar is put on the animal's neck in such a manner as not to hinder feeding and to prevent the animal from discarding it

The operating board presents a thick plank with four nails or screws in the corners, the animal is placed on the board feet downwards Each of the animal's legs is stretched out and bound with a piece of gauze to one of the nails, in such a way as to spread it prone Part of the back, at a certain distance from the shoulder-blades, is shaved clean, the hair free area being suited to the size of the prepared sleeve The rim of the sleeve is profusely smeared with glue and applied to the bare skin The sleeve should adhere without creases and gaps, being held in place with the fingers till the glue begins drying Adhesion may be quickened by blowing over the glued surface with a rubber syringe When the edges are dry, hungry ticks are let in through the open top of the sleeve, counting the number of females, males and nymphs, larvae are put in without count The brush used in transferring the ticks is washed in alcohol or lysol, so as to remove all larvae The top of the sleeve is tied up, a number tag fixed to the animal's ear and the collar put on its neck, tightening the edges with thick thread or wire After that, the animal is placed in a jar or cage, which is set on a tin tray with sides not less than 5 cm high To prevent accidental escape of detached ticks, the tray bottom is covered with water to be changed every day Blood meals are registered in the journal according to form No 2

Record Form No 2

Species and Number of Animal

Date of tick release	Tick species	No of collection	Place of collection (point date habitat host)	Number of released ticks (by stages)					
				Females		Males	Larvae	*Nymphs*	
				Hungry	Under fed			Hungry	Under fed

In attesting natural infection in ticks by feeding on guinea pigs the latter's temperature is taken at one and the same hour for 20 days, preferably in the morning, before checking the ticks beneath the sleeve and cleaning the animal The results are entered in the journal At the end of the experiment the temperature is either registered graphically or tabled according to form No 3

Record Form No 3

Temperature of Experimental Animal

Day	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Temperature									
Date									
Day	10th	11th	12th	13th	14th	15th	16th	17th	16th
Temperature									
Date									
etc									

The latter is filled in daily with the results of inspection of feeding ticks Engorged ticks, on falling off, are removed to test tubes with moistened sawdust, registering their number in the journal according to form No 4

Record Form No 4

Detachment of Engorged Ticks

Test tube No	Tick species	Origin of tick (point date habitat host)	Detachment		Number of collected ticks by stages											
					Imago						Larvae			Nymphs		
					Females											
			Species of animal	Date	Hungry	Underfed	Medium fed	Replete	Males	Total	Medium fed	Replete	Total	Medium fed	Replete	Total

The ticks on disattachment are classified as to degree of repletion which determines the duration of their subsequent development

When feeding ticks on animals of different species, the entries are made in different journals, one for each species In mass feeding on white mice, the only device required is a film collar The mouse is placed in a glass jar, the bottom of which is covered with sawdust or oats, and a feeding trough is installed on the latter Drinking water or milk is poured into a cotton wool plugged test tube which is placed upside down on the net lid of the jar The jar is put on a stand into a bowl of water whose level must be below the bottom of the jar The test tube with hungry larvae is placed open into the jar containing the mouse, after which the jar is closed with a cover of metal netting In such conditions hundreds of larvae can suck to repletion Daily inspection of the attached ticks and the jar and water bowl content serve to determine the beginning of detachment of engorged specimens, observations being continued until the last engorged tick falls off

Collection of Gamasidae, Chiggermite Larvae and Fleas in Tick Typhus Foci

Acarina of the extensive superfamily *Gamasoidea* include many temporary or permanent ectoparasites of mammals, birds and reptiles Some species inhabit the viscera and internal cavities of the body The *Gamasidae* include both predatory species and necrophagues The technique used in collecting *Gamasidae* from animals is the same as in the case of ixodid larvae and nymphs

All ticks revealed are removed from the cuvette or bag by a preparing needle dipped into 70° alcohol, and placed into test-tubes *In collecting the ticks from birds*, special attention should be given to the body, carefully going through the feathers and inspecting the beak After opening the latter, the examiner cuts out the palate, bits of which are submerged in water on a watch glass and subsequently examined from all sides with the help of a binocular lens In reptiles (lizards) the examiner should lift the scales on the body with a needle, likewise inspecting the eyelids and corners of the mouth

Gamasidae are identified microscopically, preparing the specimens as follows (N G Bregetova, 1956) The ticks are removed from the test tube to a watch glass with distilled water and washed there for several minutes On removing the water by means of a pipette, the watch glass is filled with a 5 to 10 per cent alkaline solution and covered with glass After 10 to 12 hours the ticks are washed with distilled water, then transferred into a drop of arabic gum mixture (Faure Berlese's liquid)[1] which is placed with a needle on a clean slide, and covered at the top with a clean cover glass The ticks are arranged in the drop with their abdomens upwards and downwards, to enable detailed morphologic inspection of either side of the body The content of the label is re written on the glass at the right hand side of the specimen The preparations are laid out horizontally on folders and kept from 2 hours to 2 weeks at a temperature of 60° C

Instructions on the identification of *Gamasidae* may be found in a handbook by N G Bregetova (*Gamasidae*, 1956) and the book *Rodent Ticks of the USSR*, Leningrad, 1955 (chapters by A B Lange and A A Zemskaya)

Chiggermites (subfamily *Trombiculinae*) parasitise various mammals, birds and *reptiles only in their larval stage The mites* may be distinguished under a hand lens by their red or yellow white colouring Most frequently the larvae attach in dense clusters inside the auricle, from which they are collected with an eye scalpel or sharp flat tipped preparing needle The larvae are carefully removed *from the animal and placed into test tubes with 70° alcohol* Bits of the ear damaged by the larvae are also cut off and placed in alcohol

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[1] The gum mixture includes the following ingredients 50 p distilled water, 200 p chloral hydrate, 20 p pure glycerin 30 p dry gum N G Bregetova recommends the following proportions chloral hydrate—160 gr gum—24 gr distilled water—40 ml glycerin—16 ml A glass jar is filled with water, after which the gum is added, the whole being placed for several *hours into a thermostat with a temperature* of 50° to 60° C That being done, glycerin and chloral hydrate are added, leaving the mixture in the thermostat for two more days After complete dissolution of the gum, the mixture is strained through glass wool or thin cloth within the thermostat and then kept in dark A drop of the mixture is applied on to a slide with the help of a preparing needle after which the arthropods (*Gamasidae* and *Trombiculinae*) are inserted

The larvae are prepared for identification by placing them on their backs and abdomens in a drop of Faure Berlese's liquid under a cover glass, and maintaining the preparations for several days at a temperature of approximately 60° C to render the specimen completely transparent

Fleas (Aphaniptera) are collected from animals or their burrows and nests When inspecting animals contained in bags, the examiner should first collect the fleas, for which he should carefully open the bag The fleas are extracted with soft, preferably brass tweezers, and placed into test tubes with 70° alcohol Subsequent collection is effected while combing and examining the captured animals

In the animals' natural environments, the fleas are collected in different ways Migrant fleas should be collected at burrow entrances on glue paper tubes or wads of cotton wool (V E. Tiflov, V O Potapov, 1937) To ensure the extraction of all fleas from the entire length of the burrow, up to its first turning the authors recommend the use of a long handled ladle, with which the content of the burrow is scooped into a bowl The best results are obtained by use of a Shiranovich tube, which presents a narrow pipe shaped flannel bag 4 to 5 cm wide and 100 to 150 cm long into which a rubber tube or flexible rod is inserted By swift movements, the tube is submerged for several seconds into the oblique and vertical passage ways of the burrow, after which it is taken out and inspected on a sheet The tube should be put in three times Incited by the movements of the tube, the fleas start jumping and attach to the flannel The same means may be employed to extract other inhabitants of the burrow, i e, burrowing species of *Ixodidae* and *Gamasidae*

Within domiciles, fleas are collected by inspecting bed clothes, wiping floors with pieces of flannel, leaving glue paper or saucers with water and grain on furniture and elsewhere for the night In the latter case a dead mouse is left in the saucer together with the grain (I G Ioff, O I Scalon, 1954) Fleas from rodent nests are collected by extracting them with a moist brush from bags filled with nest content, or by means of a thermoeclector

Fleas may be identified alive (I G Ioff, O I Scalon, 1954) or in transparent alcohol preparations Live fleas are put between two *thin slides in a drop of water under another slide or under a cover-*glass Alcohol samples are examined on a slide in a drop of 50° alcohol and glycerin also under a cover glass For convenience of examination *and storage, microscopic preparations of fleas are made* by placing them successively into the following liquids 7 per cent NaOH or KOH (24 hours), 50° alcohol (1 to 24 hours), 70° alcohol (the same), 95° alcohol (the same), pure alcohol (the same) clove oil (the same), after which the samples are immersed in Canadian or fir balsam, laying the insects on their right sides

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TICK-BORNE RELAPSING FEVER

Synonyms *Tick borne recurrens, tick borne spirochetosis, fièvre récurrente, ruckfall fieber, tifo récurrente, tiebre récurrente, febris recurrens, rückfall typhus, garapata disease, kimputu, spirillium fever, famine fever, tick fever, la fièvre récurrente a tiques (de l'Afrique tropicale, de Madagascar, de l'Arabie méridionale, de Sénégal, etc), Persian relapsing fever, African relapsing fever, Iranian tick bite fever, tabe gerib ghez*

GENERAL DEFINITION

Tick borne relapsing fever is an endemic tropical or subtropical disease transmitted from animals to man, with obscure or non-existent prodromic phenomena The onset is characterised by chills, temperature up to 38 or 40° C, pains, especially in the legs and lumbar regions, and headache, occasionally followed by symptoms of inacute enterocolitis The temperature curve, on the whole, is non typical for tick borne relapsing fever, attacks of various duration following irregularly In the course of the disease, the temperature curve may resemble those of malaria, brucellosis and louse borne relapsing fever Number of attacks—*from 1 to 26 (average—from 8 to 10)* The disease may continue from 1 to 2 months In patients subjected to pyretotherapy for progressive paralysis, the disease was observed to last up to 5, 6 or 7 months

Generally, the issue is non lethal

Tick-borne spirochetosis presents a zoonosis whose pathogen is easily transmitted by vectors to man, the infection thus becoming an anthropozoonosis. In the latter case, *it may likewise be transmitted from diseased to healthy* individuals, provided that the former infect hitherto uninfected ticks of the genus *Ornithodorus papillipes* inhabiting the same premises.

DISTRIBUTION

Europe: Portugal, Spain, Greece, Yugoslavia, North Caucasus, Transcaucasia.

Asia: Iran, Iraq, Syria, Israel, Arabian Peninsula, India, Western China, Central Asian republics of the U.S.S.R., Kazakhstan.

Africa: Mediterranean coast and the entire continent (south of the Sahara).

North America: South-Western Canada, western states of the U.S.A.

Central America: Mexico, Panama, Guatemala.

South America: Colombia, Venezuela; in places, Northern Brazil.

HISTORY OF RESEARCH

The first to surmise that the tick-borne relapsing fever of tropical Africa might be transmitted by ticks, was David Livingstone, and yet, despite a number of cases revealing spirochetes in the blood described by several authors, it was only in 1905 that Dutton and Todd proved definitively that the pathogen of the disease is actually transmitted by the tick *Ornithodorus moubata*. The study of the disease in question is inseparably connected with investigations on its vectors, which were conducted by Nicolle et coll. in North Africa (Tunisia), Junkovsky in Iran, and N. I. Latyshev, I. A. Moskvin, Y. N. Pavlovsky et coll., P. A. Petrishcheva and A. N. Skrinnik in the U.S.S.R.

CAUSATIVE AGENT

The causative agent is generally known to be a spirochete, the species occurring in the U S S R being called *Spirochaeta sogdianum*, described under the name by Nicolle, Director of the Tunis Pasteur Institute, and Anderson from a strain obtained in Shah Reez Abs N I Pikul, working with a strain from Ferghana, described the spirochete under the denomination of *Sp usbekistanica*, but far less exhaustively than Nicolle

The classification of spirochetes, in the broader sense of the term, has of late assumed the following form

Order *Spirochaetales* Buchanan, 1918
Family I *Spirochaetaceae* Swellengrebel, 1907
II *Treponemataceae* Robinson, 1918

The latter family includes three genera

I *Borrelia* (microorganisms easily stained with aniline dyes)

II *Treponema* (anaerobe)	Stain with difficulty, except by the Romanowsky Giemsa technique or silver impregnation
III *Leptospira* (aerobe)	

The pathogens of tick borne relapsing fever belong to the genus *Treponema* Their specific classification is extremely difficult, being founded on the specificity of the vectors, which are lice (*B recurrentis*, *B berbera*, and *B carteri*) and various species of ticks of the genus *Ornithodorus* The geographic criterion is important, as will be evident further In the latest *Bergey Manual of Determinative Bacteriology* (7th ed , 1957), the key for the identification of *Borrelia* is based on the vectors and hosts (the name of the author being appended)

Bird pathogens—*Borrelia*

Mammalian and human pathogens, transmitted by known vectors (a) human lice—*B recurrentis*, (b) ticks of the genus *Ornithodorus* (1) *O erraticus*—*B hispanica*, Pyre nean Peninsula, (2) O *hermsi*—*B hermsii*, western states of the U S A , (3) O *moubata*—*B duttonii*, Africa, (4) O *parkeri*—*B parkeri*, western states of the U S A ; (5) O *rudis*—*B venezuelensis*, Central and South America, (6) O *tholozani*—*B persica*, Iran, (7) O *turicata*—*B turicatae*, Mexico, (8) O *verrucosus*—*B caucasica*, North Caucasus, Transcaucasia, (9) O *papillipes*—*B sogdiana*, Central Asia, (10) O *tartakovskyi*—*B latychevi*, Central Asia, (11) O *marrocanus*—Morocco, Tunis, (12) O *normandi*—*B normandi*, North Africa

However, various strains of spirochetes (or *Borrelias*) obtained from different points in the southern U S S R have certain features both of similarity and distinction

The strains are similar in that they are pathogenic both for man and guinea pigs, although the "Gissar" strain is far more virulent to the latter Morphologically, the strains are indiscernible

Serologically, in the Rieckenberg-Brusin test, they prove homologous With low-titre sera, certain distinctions are apparent, serologic affinity being revealed between the strain from Tbilisi and that from North Caucasus, the strain found near Dushanbeh standing further and the strain from Gissar further still At higher titres these distinctions disappear

In cross-infection tests the said four strains prove heterogeneous Usually, immunity develops only in regard to a homologous strain, but there are some exceptions, however few. All four strains belong to the same group

Although cross immunity tests reveal only individual, but not specific or varietal distinctions, there are, nevertheless, certain immunological distinctions apparent between the various strains of spirochetes, which may prove of practical epidemiological importance for man

An exact definition should be agreed upon for heterologous and homologous spirochete strains. An individually isolated strain from a vertebrate host or tick vector and its subsequent generations obtained by passages through a laboratory animal of the same species, may be regarded as homologous Heterologous strains are those isolated from animals not only of different geographic origin (including different settlements in the same region), but also from animals of different biotopes occurring in the same locality, e g , the burrows of porcupines, rats and gerbils, cattlesheds, etc

On the basis of experiments described in literature, the following general statement may be formulated Spirochete strains obtained from the same geographic point (locality), as a rule, do not cross immunise, thus depriving the cross-immunity test of the significance ascribed to it in the specific identification of spirochetes (Y N Pavlovsky and A Y Alimov, L P Delpy and A Rafyi) Apparently, bacteriologists should evolve other more effective indices in order to classify *Borrelia* and develop a reliable technique for cultivating these microbes

The problem of tick-borne relapsing fever requires further research To ensure effective accumulation of more accurate comparative data, a unified methodology should be maintained in the collection and storage of ticks of the genus *Ornithodorus*, in order to preserve the specimens *in vivo* with a view to (a) elucidating the possibility of their spontaneous infection with the causative agents of tick-borne relapsing fever, (b) staging experiments on the stage-to-stage and transovarial transmission of these pathogens in ticks, and on the length of maintenance of the pathogen in tick organisms Substantial proof is available showing that *Ornithodorus papillipes* are capable of surviving long periods of starvation (up to 14 years) and maintaining spirochetes during these periods (*Y N Pavlovsky and* A N Skrinnik) (For collection and maintenance of live *Ornithodorus* ticks, see further)

ORNITHODORUS TICKS—VECTORS OF SPIROCHETES IN THE U S S.R

Acarina of the genera *Ornithodorus* and *Argas* belong to the family *Argasidae* These arthropods as well as the pasture ticks of the family *Ixodidae* are referred to the superfamily *Ixodoidea*

For specific identification, the arthropods are examined from above and below through a binocular microscope under directly falling light In case the tick is alive, it should be placed into a small Petri dish approximately 5 cm in diameter, covered at the top with a piece of a slide to prevent the arthropod's escape If the tick happened to be in alcohol, it is previously dried on both sides with filter paper The dry tick should be handled with care, so as not to break off its legs The tick must also be examined from the sides, for which purpose it is inserted in a groove made in a cork stopper, or otherwise, a layer of plasticine is applied to the slide, a depression of the required size being made in the middle With some experience, in field conditions, preliminary identification of male and female ticks and large nymphs may be done with the naked eye

During specific identification, the following external features are noted structure of chitinous cuticle, body outline, arrangement of discs to whose inside the dorso-ventral vertical muscles are attached[1] From the ventral side, the combination of mouth parts, or capitulum, are examined The latter is surrounded with isolated, partly mobile cheeks, which may be solid or digitate The presence of the cheeks is correlatively associated with postanally located crosswise grooves or ridges of the chitinous cuticle—the anomarginal and transverse postanal ridges, which together form a figure of the cross Ticks without cheeks are likewise deprived of these markings The genital

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[1] The discs are well visible on a live tick appearing as transparent discs when the tick is examined in strong transient light the rest of the chitinous cuticle looking black

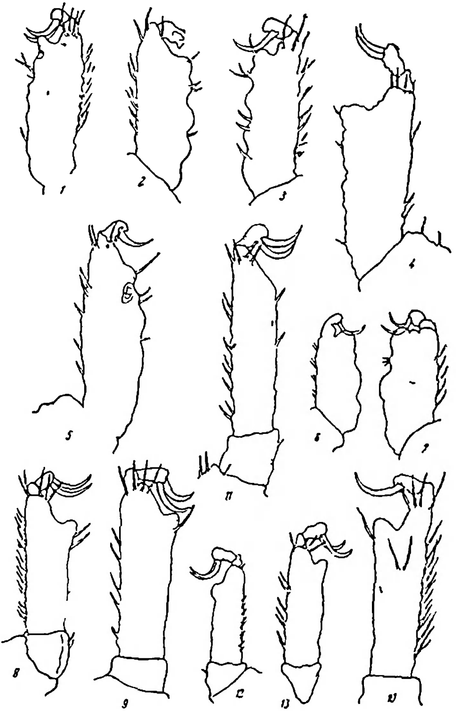

Fig 12 Tarsi of *Ornithodorus* (Y N Pavlovsky)

First pair 1—*O papillipes* 2—*O verrucosus* 3—the same male 4—*O cholodkovskyi* 5—*O nereensis* female 6—*O tartakovskyi* female 7—*O jacobsoni*. Fourth pair 8—*O papillipes* 9—*O verrucosus* female 10—the same male 11—*O nereensis* 12—*O tartakovskyi* 13—*O jacobsoni* orifice in the female lies between two transverse semiovate shields, one of which in the male is oval

An important specific sign is the shape of the superior margin of the tarsi in the first and fourth pair of legs, which for better microscopic examination should be separated from the body (Fig 12)

As evident from the appended table, the combination of the mentioned features permits identification of the principal species of *Ornithodorus* included in the fauna of the U S S R (the Caucasus, North Caucasus, Central Asia)

Classification of Ornithodorus Ticks of the Fauna of the U S S R.

A number of authors consider the widespread species O *papillipes* Birula to be synonymous with O *tholozani* described from Iran Yet, judging by its pictures, the latter has been described inaccurately, some of its features being confused with O *lahorensis* For this reason, we do not employ the term O *tholozani*

M V Shtrom, on the basis of Warburton's extremely sketchy information on O *asperus*, which he described from a single specimen obtained in Mesopotamia, identifies this species with O *verrucosus* from North Caucasus described by N O Olenev, D N Zasukhin and G K Fenyuk (1934) The former assumption, however, is erroneous Both species have their separate identity, of which we were personally convinced on examining O *asperus* in the Berlin Zoological Museum It is equally wrong to unite O *lahorensis* and O *canestrinii* into one species, since these are easily distinguished by the structure of the dorsal chitinous covering, which in O *lahorensis* is stellate and in O *canestrinii* roughly ridged The same author unnecessarily rebuilds and complicates the generic and specific nomenclature of *Ornithodorus* ticks, splitting the genus into a whole series of independent genera It should be added, also, that O *crossi*, described from India, is a synonym of O *papillipes*, which was confirmed by G Nuttall

Collection of Ornithodorus Ticks

The operation requires the following equipment

(1) shovel, tablespoon, broad, strong knife,

(2) white coloured cuvette and piece of white oilcloth 0 5 sq m in size,

(3) sieve 25 to 30 cm in dia , with a mesh not less than 2 mm in size, the sieve may be replaced by a screen comprising an oblong wooden frame with a side from 5 to 7 cm high and an area of 25x40 cm covered with a wire net of at least 2 mm mesh size,

(4) tweezers with thin ends (not surgical), medium size paint brushes,

(5) set of flat bottomed test tubes 1 5x7 to 8 cm, or chemical test tubes, containing strips of filter paper bent treble, lengthwise, each test-tube should be tightly closed by a twisted (preferably, gauze-covered) cotton wool plug the test tubes are placed into oval shaped tins of suitable size in such a manner as not to break during movement or work, long test tubes are placed into a cartridge belt for 12 15 tubes made of a double layer of cloth, which is folded and tied with cord Well suited for the purpose are flat bottomed test tubes for faeces samples with the plug and attached spoon replaced by a cotton wool stopper,

(6) field log book for keeping records, label tags of thick white paper, soft black pencil,

(7) bag for all articles except the sieve, extremely desirable is a photographic camera for taking pictures of the terrain and biotopes of *Ornithodorus* ticks,

(8) wick lantern or electric torch for work in dark lairs and premises

Collection Techniques

Investigations in buildings In non domestic structures such as cattlesheds, a frequent feature are cobwebs suspended from the beams and ceiling These may reveal tick cuticles discarded during moults, which are shaped as elon

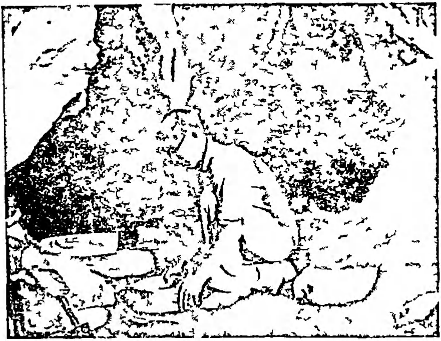

Fig 13 Entrance to grottoes in steep river bank used as winter shelter for cattle Sifting of grotto content yielded abundance of ticks *Ornithodorus* Mangyshlak

gate, trough like whitish leaves 3 to 5 mm long Such cuticles are to be found in abundance (our own observations in Khorossan northern Iran) The cobwebs should be removed and placed into test tubes In highly infested premises, the ticks may be found sitting directly on the walls (as observed in the same locality)

Most frequently, the ticks may be observed in depressions at the foot of walls, in rodent burrows and wall fissures which are widened with a knife as well as in the intervals between stones in the walls, under layers of earthen stuccowork which are broken off piece by piece The content of fissures and depressions is scooped out with a shovel or spoon and put into a sieve, the same being done with the refuse at the foot of the walls The sieve is care fully carried out into the light, where it is shaken over an oilcloth which should be spread out on the ground. The finer particles penetrating through the sieve are thoroughly examined, extracting everything observed to move by scooping up the ground with the edge of a test-tube (or paint-brush), since the object may prove to be a larva or first nymphal stage of *Ornithodorus*. After sifting, the remaining substratum is inspected for ticks, which for a time may remain motionless. The ticks are taken up with tweezers and put into a test-tube with a certain amount of fine substratum.

All ticks collected from a single room are kept together in a separate test-tube; the same applies to larger ticks extracted from pieces of stucco broken off from the walls The respective notes are made in the log-book and on labels which are forthwith inserted in the test-tubes. If dead and dry ticks should occur, they are also collected in a separate test-tube, care being taken not to break off the legs.

Fig 14 Base of wall in old fortress Pil-kala (Kara-Kalpak Autonomous S S R). Abundant yield of *Ornithodorus papillipes* from burrow.

Fig 15 Burrow of *Spermophilopsis leptodactylus* in sand dunes near Ashkhabad in winter Foreground—soil excavated from burrow containing *Ornithodorus tartakovskyi*

A chart of all premises on the grounds is drawn up, including the domicile and services, marking with crosses the places where ticks were revealed

Collection in natural biotopes The content of early spring burrows and holes made under stones, is scooped out with a shovel or spoon and strained through a sieve as described above In large caves ticks are caught by attracting them with one's own body. The collector lies down on a white sheet spread on the ground in such a position as to leave enough of the sheet free all around his body After a while, another worker with a lantern inspects the edges of the sheet, which often reveal *Ornithodorus* ticks lured by the scent of the human body. The ticks are collect-

Fig 16 Entrance to porcupine burrow infested by *Ornithodorus papillipes* Discarded spines seen in foreground Burrow may serve as natural focus of tick borne relapsing fever South Eastern Central Asia

ed by means of tweezers In winter, *O lahorensis* may be discovered on camels goats and sheep which serve them as warm shelters for hibernation in the South

It is desirable likewise to photograph all biotopes of *Ornithodorus* Some familiar object should be placed at the entrance to a burrow or lair to offer an idea of their size It is also desirable to photograph the general view of the terrain settlement and domicile Each collection should be provided with a label written in soft pencil on a piece of thick paper, e g

Kulab wall of cattleshed
205 1950 A Petrov

Near Kara Kala (Turkmenia) porcupine burrow among hillocks 157 1952 N I Ivanov

Near Shakhrood village of Kelyata stone pen on wasteland between stones 107 1943 Alekseyev

The completest data are available on the biology of O papillipes

Egg-laying occurs towards the end of summer and the beginning of autumn, which is also observed in ticks maintained in laboratories far from their range of distribution The egg hatches into a six legged larva feeding on the blood of small animals Moulting results in the emergence of nymph I, which after suction moults into nymph II, the latter in the same manner transforming into stage III Nymphs III, in their turn, moult into the fourth nymphal stage, males as well as females, if the family is sufficiently large On repletion, nymphs IV moult into nymphs V, females and males, and, finally, nymphs V likewise moult into males and females A distinctive feature of the process are the three successive batches of males and females appearing in the life span of a numerous family.

The same pattern is observed in the biology of other species of *Ornithodorus* In nature, ticks feed on the blood of mammals, birds, turtles, sand dragons lizards, toads, and, on occasion, man The total length of metamorphosis depends upon frequency of blood meals and resistance to starvation Cases were observed when nymphs and mature specimens of O *papillipes* starved for from 10 to 11 years Extremely hardy in this and other respects in the embryonic stage is O *lahorensis*, and to a progressively diminishing degree—O *verrucosus*, O *tartakovskyi*, O *nereensis*

The following are the approximate terms of metamorphosis for O *papillipes* maintained in the laboratory The female lays up to 60 eggs, which, under equal conditions, do not all produce larvae At temperatures from 15° to 18°C the larvae hatch from the eggs in 11 to 30 days, and at room temperature can starve up to 15 months Blood meals continue from 8 to 60 minutes At 26°C, and a definite level of humidity, the larvae have a life span of 15 to 30 days, the first nymphal stage from 17 to 28, the second from 20 to 31, the third from 24 to 25, and the fourth from 43 to 120 days and more Ticks of both sexes have survived in laboratory conditions for 6, 7, 8, 9 and 12 years, not counting the part of their life prior to collection It is highly probable that the entire life cycle of O *papillipes* may last up to 14 or 15 years, or perhaps even longer (A N Skrinnik)

Famine resistance in different metacyclic stages is illustrated by the following findings of our laboratory Nymphs I starved 18 months, nymphs II—2 years and 5 months, nymphs III—3 years and 5 months, and nymphs of undefined stages—6 years and 3 months (A N Skrinnik) Apparently, the life cycle of O *verrucosus* is similar to that of O *papillipes*, but as yet has been traced only to the males and females derived from nymphs III and to nymphs IV According to observations by A N Skrinnik on O *tartakovskyi* from the Mangyshlak Peninsula, males and females may appear after the moulting of nymphs II We, personally, have likewise encountered extremely small females in burrows near Naarli (Turkmenia)

The biology of the O *lahorensis* group, apparently, differs from the above described

Ecology of Vectors

Temperature drops render the ticks semi- or *completely* immobile The ticks endure decreases of temperature to —15°C, and, although slowly, will suck blood at +5 C, being capable of infecting guinea pigs at *this temperature (Y N Pavlovsky and A N Skrinnik) According to* N I Latyshev and A P Kryukova, burrow ticks (O *tartakovskyi)* inhabiting the burrows of susliks and gerbils, find optimum conditions for existence in August October, when the burrow temperature is +24 to +26° C and relative humidity from 75 to 90 per cent, while outside the burrow the respective indices vary from +15 to +42° and 1 to 72 per cent In cattlesheds, at different heights from the floor, there are zones whose temperature approaches that of the natural biotopes of O *papillipes*, which serves to explain the infestment of such premises, all the more so that the required hosts are present as well

O papillipes was often found alive in winter under layers of earthen stucco Apparently, they may survive for considerable periods with a lack of air, which has been proved in direct experiments (E Sosnina)

Special experiments staged by the author and I K Teravsky (1956) in glass chambers with two exhaust pipes, gave the following results in regard to the maintenance of O *papillipes* (males and females) without air and in various gas media

Experimental conditions	Earliest evidence of toxic effects of gas or lowered ambient pressure		Period in which ticks perished (days)
	Minimum	Maximum	
Vacuum res press 8 mm m c	40 to 48 hrs	7 days	4 to 17
Hydrogen	30 to 40 hrs	3 days	4 to 5
Nitrogen	7 to 10 days	16 to 20 days	20 to 25
CO_2	3 to 4 hrs	2 to 3 days	4 to 10
H_2S	8 to 10 min	12 to 15 min	1 to 2

O *papillipes* are adapted to darkness, but may also be present in dry refuse or earthen floors with lighting varying from 10 to 70 lux At cattleshed doors the lighting may reach 1000 lux, and in the sun—over 20,000 (Neuymin) However, adaptation to dark biotopes does not prevent the ticks from crawling out to better lighted places and attacking man at any hour of day or night

Storage of Collected Ticks

Ticks are collected for two purposes (1) establishment of the presence of ticks g *Ornithodorus* on the site of work, specific identification and faunistic collections, (2) preservation of ticks *in vivo* for (a) study of biology, (b) testing for spontaneous infection with the pathogen of tick borne relapsing fever, (c) experiments (particularly, on stage to stage and transovarial transmission of the pathogens of tick borne spirochetoses and rickettsioses), (d) establishment of a vivarium for long term observations on *Ornithodorus*, as well as maintenance of pathogen strains of tick borne spirochetoses for experimental, and, possibly, pyretotherapeutic needs

In the first case, the material used are dead (dry) ticks with more or less intact bodies or else live ticks fixed with 70° alcohol (or 4 per cent formalin, which is worse) The material supplied with the respective labels is kept in 50, 100, and 150 ml jars with tight fitting stoppers Minute objects, such as larvae and nymphs, are stored in small test tubes filled with alcohol, each stage separately, with the necessary labels applied, all in a common tin container, separating the test tubes with hygroscopic cotton wool The chitinous cuticles discarded after every moult are preserved in the same tin

To preserve live ticks in a vivarium, collected specimens from each test tube are transferred without the substratum to 50 ml Erlenmeyer flasks, inserting a filter paper disc on the bottom and a corrugated strip of the same paper on top The flask is plugged with a gauze covered cotton wool stopper A clearly written label is placed into the flask with the inscription facing the glass, and a tag glued outside with the number of the given collection The flasks are placed into wooden trays 40x40 cm in size with sides 6 cm high. Vertical grooves are made on the inside of the walls to insert strips of plywood for partitioning the rows of flasks On the face side, the walls are inscribed with the geo graphic point of collection or species of the ticks, depending on the purpose of the collection. The trays with the flasks are kept in a dark cabinet. When kept indoors in the southern summer, the ticks may be killed by the excessive dryness of the ambient air. To prevent this, the flasks should be moistened periodically by applying 2 or 3 drops of water from a pipette to the filter-paper inserted in the flask, taking care not to overmoisten it. Sometimes, it may prove necessary to keep the collection on cold premises. In northern regions, it will suffice to keep the ticks at summer room temperature, maintaining, likewise, a constant level of humidity. For experimental purposes, ticks should be kept in a thermostat with a permanent temperature of 25°C or more.

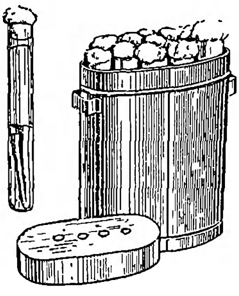

Fig. 17. Left—test-tube with paper for storing *Ornithodorus* ticks; right—tin container for test-tubes (Y. N. Pavlovsky).

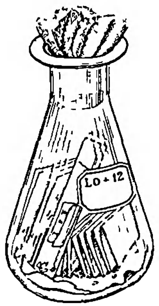

Fig 18. Flask for live *Ornithodorus* (Y. N. Pavlovsky).

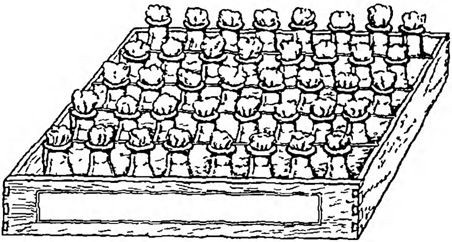

Fig 19 Storage of live *Ornithodorus* ticks. Trays with flasks to be inserted in cabinet.

Ticks to be maintained alive are fed once or twice daily on guinea pigs or other laboratory animals.

The content of the flasks should be regularly inspected. Dead ticks and discarded cuticles should be removed to small test-tubes stopped with cotton wool, placing them in the same flask and recording the time.

Altogether, three journals should be kept, the form of which is described hereunder.

1. Catalogues of tick lots with reference to habitats of collection, containing regular entries on operations carried out with the respective lot in regard to feeding for natural infection tests (with reference to the given lot's register number in the special feeding journal described further). The tick catalogue should be kept with the utmost care and regularity, a separate page being allotted to each flask. This page should contain the initial number of ticks, dates of death, moistening and feeding with reference to the number in the blood meal and infection test journal; moulting, oviposition and occurrence of signs of copulation (discarded spermatophores).

In the laboratory, different species of ticks survive for different periods Hardiness in diverse species may vary The American *O hermsi* reproduces freely *O lahorensis* readily lays eggs which hatch into larvae *O papillipes* and *O verrucosus* are hardy enough, *O tartakovskyi* more exacting, the metamorphosis of the Iranian *O canestrinii* is extremely distended *O nereensis* also requires more care

In the course of time, the filter-paper is stained with dry faeces, and must be replaced, being subjected to careful examination All inspections of flask content should be made in a white photographic cuvette, putting back all ticks which happen to fall or crawl out When extracting the flask stopper, the worker should check whether the ticks have not gathered directly under it, the flask should be shaken and the stopper removed with due care, so as not to crush the ticks

2 Journal for registering blood meals, noting the lot number, with reference to the numbers of experimental guinea pigs registered in the third journal

3 Journal with register numbers of guinea pigs used in feeding ticks for different purposes, with note of the lot number

For convenience of use, all three journals should be furnished with complete cross reference so that general observation and experimental data may be looked up when applying to any of the journals

Feeding of *Ornithodorus* Ticks

As a rule *Ornithodorus* ticks feed quickly The length of a blood meal may vary from tens of minutes to several hours depending on time of starvation, period elapsed after moulting, stage and phase of metamorphosis, rate of digestion of earlier obtained blood, which in turn depends on temperature Due to the brevity of the process, ticks are fed on animals under direct observation If the blood meal is purposed to check spontaneous or experimental infection, it is conducted on a reactive laboratory animal, depending on the expected kind of pathogen of tick borne spirochetosis

Extensive experimental evidence shows that O *papillipes* and O *verrucosus* should be fed on guinea pigs and O *tartakovskyi* on white mice

Feeding procedure The guinea pig is tied by its legs to a wooden board with four nails in the corners, which is then placed into an enamel trough 30x40 cm in size The fur on the guinea pig's abdomen is sheared close and a wide test-tube or open cylinder 4 cm in dia and 5 cm high containing ticks is applied to the abdomen The cylinder or test tube is held with the left hand, putting the ticks inside with the right All ticks should be allowed to attach those refusing to do so being put back with tweezers into the flask When all ticks attach, the test-tube or cylinder is removed, care being taken, however, that the guinea pig should not throw off the ticks until the blood meal is finished At the end of the meal the guinea pig is untied and replaced into its cage

When feeding larvae or small nymphs on white mice, the mouse may be put into a wire cage small enough to prevent movement The cage is put into a large cylinder into which hungry larvae are released, the cylinder being covered at the top with dense wire netting On falling off, replete ticks are picked up on a soft paint brush and put into a flask for moulting

Tests for spirochetal infection On the fourth day pending a blood meal, blood is taken from the guinea pig's ear by means of Francke's needle, etc Three thick films are prepared of the extracted blood on two slides inscribed with the respective number in order In summer, the preparation should be dried under a glass bell to keep off flies The films are stained with the Giemsa-Romanowsky solution (1 or 2 drops of the dye at a temperature not lower than 16 to 17°C, to 1 ml of distillate or rain water) The slide with the film is treated the same as in tests for malaria. The procedure takes 30 to 40 minutes, after which the slides are washed in water, air-dried and immersion-examined under a microscope. Dark-field microscopy of fresh blood may be employed as well Since spirochetes in the thick film test do not appear in great numbers, and do not occur at each view, the preparation should be examined with redoubled caution.

If thick film microscopy does not reveal spirochetes, this alone does not signify their absence in the guinea pig's blood. If daily blood tests during 15 or 20 days do not isolate spirochetes, blood taken from the guinea pig under test is introduced into the eye-lid or nasal cavity of another. The latter is similarly subjected to the thick film test, which is repeated for 4 to 20 days. If spirochetes are still non-apparent, the guinea pig may be considered uninfected In high-precision tests, a second series of tests with blood from the latter-mentioned guinea pig is required.

The following is an example of the entries to be made in a tick feeding journal.

EXTRACT FROM TICK FEEDING JOURNAL

Tajikistan, 1951

Varzob district, kishlak (village) of Gazhni Cattleshed, Aug 23, 1951 B Skvortsov 48 ticks

Checked Oct 26, 1951. 35 ticks alive, remainder dead

Oct. 30, 1951. Fed on healthy guinea pig No 69—1951 to test natural infection—32 ticks sucked blood

2 M, 2 F, 28 N, 3 nymphs did not attach *Guinea pig infected*

June 2, 1953. Fed on guinea pig No 64—1952 (used in earlier experiments, but not diseased) 30 ticks sucked blood 5 M, 3 F, remaining 5 ticks died *Guinea pig infected*

July 13, 1953 Flask observed to contain eggs

Oct. 26, 1953 Larvae extracted

May 25, 1955 27 ticks alive, 3 dead Number of discarded cuticles 21. Fed on guinea pig No. 35—1954 (previously used 4 times to feed uninfected ticks) All 27 ticks sucked blood including 12 M, 6 F and 9 N. *Guinea pig infected*

Jan 15, 1957 20 ticks alive, 7 dead Number of discarded cuticles 9 Fed on guinea pig No 23—1956 (earlier used 7 times for feeding non infected ticks) All twenty sucked blood, including 10 M and 10 F

Nov 25 1957 Flask contains many live larvae Guinea pig infected

Feb 28, 1959 7 ticks alive, 13 dead Fed on guinea pig No 9 a—1958 (previously used ten times for feeding ticks) 2 M and 5 F

Guinea pig's blood tested daily from 5th to 17th of March Spirochetes not revealed

EXTRACT FROM EXPERIMENTAL GUINEA PIGS JOURNAL

Guinea pig No 24—1958

Feb 19, 1958 Used in natural infection test to feed *O nereensis* 6 T, 1958, Turkmenia found in burrow under stone, Oct 23, 1958 173 ticks applied, 142 sucked blood

Blood tests Nov 25, Nov 26 Nov 27, Nov 28, Nov 29 Dec 1, Dec 2, Dec 3, Dec 4 Dec 7, Dec 8

Spirochetes absent on all occasions

Dec 30 Used to feed *O papillipes* lot 70, found in Kirghizia Osh under a wall of a house, Dec 10 1949 25 ticks applied, all sucked In 1949 these ticks had infected a guinea pig, 4 subsequent blood meals produced negative results

Blood tests Jan 5, Jan 7, Jan 10, Jan 12, Jan 14

Spirochetes absent on all dates

March 11, 1959 Used to feed *O papillipes*, 8 b—1945 13 ticks applied, all sucked Bred in laboratory from Iranian ticks (33—Iran) infected with 'Kara Kalpakia" spirochete strain

Blood tests March 16, March 17, March 18, March 19, March 20 March 21, March 24

Spirochetes present on all occasions

March 24, 1959 Guinea pig died

NEUROTROPICITY OF SPIROCHETES

A characteristic feature of the spirochetes of tick-borne relapsing fever is their neurotropicity, eventually, they concentrate in the brain, as observed in guinea pigs The said process may be associated with their disappearance from the peripheral blood Therefore, when looking for spirochete-carriers among animals, apart from testing their blood for apparent or latent spirochetosis, it is necessary to grind the cerebral substance and inject the suspension into a guinea pig's abdominal cavity with subsequent thick film staining tests The presence of spirochetes in the brain along with their absence in peripheral blood, should be regarded as the termination of carriership Notwithstanding, the animal under test should be set down as a zoological species in the list of reservoirs of tick borne spirochetosis, since the spirochetes attain the cerebrum from the peripheral blood

INFECTION OF MAN AND GUINEA PIGS BY SPIROCHETE CARRYING VECTOR TICKS

Man may contract tick borne relapsing fever after his blood has been sucked by two infected females (as in pyretotherapy) or two nymphs (I or II) (author s observation on himself) Experiments on guinea pigs confirmed that transmission may be effected during the first 1 5 minutes of suction by one tick

Experimental series have demonstrated that a vector, though known to be infected, does not invariably infect a guinea pig while sucking its blood Thus, a male tick fed successively on 5 guinea pigs (transferred from pig to pig with interruption of blood meals), infected only the second guinea pig in the series, not affecting the first, third and fourth, and not attaching to the fifth In another instance a nymph did not infect the first and third guinea pigs, but did infect the second Neither is one and the same infected tick apt to inflict regular infection every successive year Thus, an infected nymph caught in Ferghana in 1928, infected a guinea pig in 1935, but failed to do so in 1937, in the same year it moulted into a male which succeeded in infecting a guinea pig Another example nymphs of *O papillipes* infected on October 9 1935, transmitted the disease to guinea pigs in the course of metamorphosis in November and December of 1935 and January 1936 In March 1936, when in the fourth nymphal stage they failed to cause any marked spirochetosis, while 7 and 15 months later they did

The above quoted examples present merely the external aspects of the phenomena, analysis of the causative factors being the task of the future Nonetheless, it is already clear, that the possibility of latent spirochetosis developing after re inoculation of blood from unafflicted to healthy guinea pigs, should be ruled out

TRANSOVARIAL TRANSMISSION

It is a long established fact, that in O *moubata* the progeny of an infected female are able for several generations to transmit spirochetes to laboratory animals The explanation is that during the pathogen's circulation in the vector organism, the developing egg is infected through the walls of the ovary, while during the embryonic period, the salivary glands of the larva are likewise inseminated with pathogens This process was similarly observed in O *papillipes* (Y N Pavlovsky and A N Skrinnik) Only part of the infected females produce infected progeny, and not all the larvae hatched are able to transmit the disease However, with the progress of metamorphosis, the incidence of transmission somewhat increases Transovarial transmission of spirochetes in O *verrucosus* was reported by S Kandelaki

With O *papillipes*, each blood meal on man continues for 30 to 40 minutes A noteworthy point is the minimum suction time necessary for the transmission of spirochetes to the recipient Experiments with ticks given interrupted blood meals on four guinea pigs, demonstrated that infection may take place already in the first 1 5 minutes after attachment This period, however, may vary for different metacyclic stages, or owing to diverse circumstances Moreover, an O *papillipes* known to be infected, does not transmit the pathogen invariably Thus, a male fed on 5 guinea pigs did not infect the first (in a 30 sec blood meal) infected the second (one min) failed to infect the next two (2 and 7 min) and did not attach to the fifth Apparently, such variations depend on the state of the organism of the animal being infected These circumstances should be borne in mind in assaying experimental results

NATURAL FOCI OF TICK BORNE RELAPSING FEVER

Tick borne relapsing fever was first described as a disease of man in tropical Africa In the described cases, ticks were found not only in the native dwellings, but even in the road dust The discovery of the disease in other countries (Iran, etc) was likewise associated with human dwellings, but analysis of published works also testified to the incidence of ticks of this genus in rodent burrows (C *Nicolle et coll, Tunis) Similar observations were made* in Turkmenia (Y Vlasov, P Petrishcheva, Y N Pavlovsky) and other republics of Central Asia, U S S R

Ticks were discovered in the burrows of different animals, in caves, grottoes and abandoned buildings Natural infection tests were often positive The species known as hosts for these ticks being numerous, wild animals were in many cases tested for incidence of pathogens and sensitivity to experimental infection

Nicolle (Tunis), when inspecting rodent burrows, revealed the presence of *Ornithodorus* ticks spontaneously infected with spirochetes of tick borne relapsing fever The range of the animal hosts of *O papillipes* is extremely wide, in other tick species, especially those inhabiting burrows, it is more limited Ticks of this genus may be the components of biocenoses in different biotopes In view of that, investigations of natural foci of the disease in question should include spontaneous infection tests (by the described procedure) both of the ticks obtained in nature and of wild animals *The latter should involve blood tests for pathogen* incidence, but owing to the possibility of latent incidence, they should be augmented by injections of cerebral and splenic suspensions into guinea pigs with subsequent investigation of the latter's blood.

With the help of the said procedures the following reservoir species were revealed: bats *Rhinolophus ferrumequinum* (Takhta-bazar, Turkmenia, N. I. Latyshev and T. Pozivay, 1936) and *Pipistrellus pipistrellus bactrianus* (near Dushanbeh, M. N. Keshishyan, 1936); rodents: large gerbil *Rhombomys opimus* (Takhta-bazar, Serakh district, Turkmenia, N. I. Latyshev, T. Pozivay, 1936, spirochetes pathogenic to man), gerboa *Alactaga elater* (near Baku, P. P. Popov, I. Akhundov, 1941); Turkestan rat *Rattus turkestanicus* (cave near Dushanbeh, N. I. Latyshev, 1936; M. N. Keshishyan, 1935; Khorog, Western Pamir, G. Y. Zmeyev, 1940); domestic mouse *Mus musculus severtzovi* (Chirchikstroy near Tashkent, M. S. Sofiev, D. Okhrimenko, 1936); vole *Microtus socialis* (near Baku, P. P. Popov, I. Akhundov, 1940); Carnivora: *Canis familiaris* (Old Bukhara, L. M. Isayev). It is very likely that the spirochetes of tick-borne relapsing fever thus revealed may be systematically non-identical.

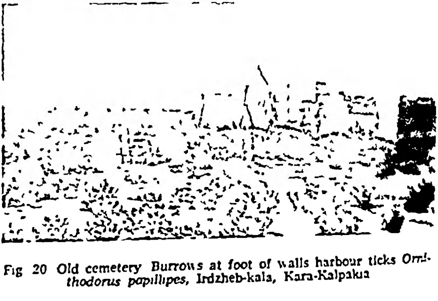

Fig 20 Old cemetery Burrows at foot of walls harbour ticks *Ornithodorus papillipes*, Irdzheb-kala, Kara-Kalpakia

Fig 21 Excavation of rodent burrows in Tigrovaya ravine Southern Tajikistan

It is likewise necessary to determine the susceptibility of different species to tick borne relapsing fever by employing them to feed spontaneously infected vector ticks (*O papillipes*) The author successfully infected a hedgehog (*Parechinus hypomeles*) from Kara-Kala (Turkmenia) by feeding ticks obtained from a cave near Dushanbeh G V Serdyukova managed to infect a jackal (*Canis aureus*) through ticks

The susceptibility of animals to infection was determined likewise by inoculating them with infected blood from animals of another species Apart from the species mentioned earlier, the following also proved susceptible short tailed bandicoot rat *Nesokia* (strain ' Western Pamir' , Y N Pavlovsky and I K Teravsky), grey hamster *Cricetulus migratorius* (the same), lemming *Lagurus lagurus* (strain ' Tbilisi ', Y N Pavlovsky and P P Perfilyev) fat dormouse *Glis glis* (strain "Saganlug' , S P Kandelaki) red tailed gerbil *Meriones erythrourus* (strain "Kirghizia ,

Y. N. Pavlovsky and A. Y. Alymov, strains "Kerghez", Azerbaijan, P. P. Popov, I. Akhundov), etc.

Among the vectors of tick-borne relapsing fever singled out in our wild fauna, three species—*O. verrucosus*, *O. nereensis* (with a closely related species from jerboa burrows in Armenia), and *O. tartakovskyi*—inhabit exclusively in nature, their presence in human dwellings and economic biotopes as yet being unrevealed. The basic *vector species (as regards epidemiological importance)*—*O. papillipes*, inhabits both natural and economic biotopes either in the complete absence or direct vicinity of man. As in other species of *Ornithodorus*, its range includes Central Asia, the arthropod presenting a natural (non-introduced) member of the local fauna. Hence, basically, none of the tick species serving as vectors for tick-borne relapsing fever, owe their existence to the presence of man.

Fig 22 Desert terrain with gerbil burrows serving as natural foci of Pendinski ulcer.

In natural biotopes unaffected by man, vector ticks were often found carrying spirochetes virulent for humans Obviously such ticks could *in no way* receive the pathogens from diseased people

The cited cases of spirochete incidence in a number of wild animals coexisting with vector ticks in common biocenoses, give cause to regard tick borne relapsing fever as a zoonosis (Y N Pavlovsky) Foci of the disease have long been existing in nature owing to the circulation of spirochetes between ticks and their alimentary hosts, the importance of man as a link in the food relationships of such foci being null The abundance of alimentary hosts of *O papillipes* offers sufficient grounds to speak of the *diversity of its feeding relationships These relationships* as it were, present the routes for the transmission of the spirochetes (simultaneously with the feeding of the tick on the recipient host), or inversely, for the tick's reception of spirochetes from the donor

In their genesis and existence, natural foci of tick borne relapsing fever are completely independent of man, but are liable to become a source of infection for non resistant people when the latter penetrate into the active sphere of such foci and are attacked by vector ticks This explains the cases of infection of man in unpopulated localities after sleeping in caves, grottoes, under overhanging rocks, near the lairs of wild animals, etc

Natural *foci of tick borne spirochetosis* are usually stable and confined within a definite epidemiological sphere of *influence which follows, primarily, from the aforesaid* features Under definite circumstances such foci may irradiate into man made economic biotopes and originate new foci closely bound with environments directly or indirectly dependent on man i e , on factors of anthropurgic nature[1] This process is associated with the establishment of permanent settlements near or within a focus of tick borne relapsing fever The topographic conditions may be such that the burrows containing the vector ticks may become included in the settled area or be shifted by rodents migrating to more "bountiful" and hence more alluring economic biotopes The possibility of the latter, however, is restricted by the fact that *O papillipes* spend an extremely limited time on their alimentary hosts

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[1] The term anthropurgic has been proposed for factors directly or indirectly concerned with the life and activity of man

Since both the rodents (rats or gerbils occasionally becoming as it were, commensates) and the ticks migrating into the direct vicinity of man, may be reservoirs of the spirochetes of tick borne relapsing fever (obtained in the natural focus), a new focus of disease springs up In the new ecologic circumstances the latter's existence is likewise maintained by man who occasionally becomes a link *in the transmission chain of the pathogen Besides this* pathophysiological relationship, an important part is played by man's economic activity itself, i e , by the character of the buildings he erects, conductive, in certain cases, to the existence of ticks, inadequate sanitation of domestic premises, maintenance in the latter of domestic animals appearance of conditions favouring propagation of commensate pests (rats, mice, etc) and the latter's existence in the neighbourhood of man, etc , etc

In view of the said relationships, special consideration should be given to the part played by domestic animals in maintaining the circulation of the spirochete of tick borne relapsing fever in the direct vicinity of man The importance of domestic animals as spirochete reservoirs depends *on two circumstances (1) the possibility of vector ticks* feeding on animals, (2) the susceptibility of animals to infection The first circumstance depends upon (a) the *willingness of vector ticks to feed on the given species* of animal (b) the possibility of contact between the tick and its alimentary host

Various domestic animals have been subjected to experiments involving infection with the spirochetes of tick-borne relapsing fever In the experiments of numerous authors (I A Moskvin, A Y Alymov, P P Popov, S P Kandelaki et al), dogs proved susceptible An attempt in Khorog (Western Pamir) to infect a young lamb and kid through the bites of O *papillipes* undertaken by G Zmeyev (Pamir expedition of the Tajik branch of the U S S R Academy of Sciences), gave negative results The experiments staged in Moscow by ourselves in collaboration with A Cheskis (1942) involving the infection of young pigs with a strain of spirochetes isolated from O *papillipes* in Ferghana by injecting pathogen carrying blood into the nostrils and conjunctiva, as well as subcutaneously and intraperitoneally, caused neither apparent nor latent spirochetosis Other authors have likewise seldom succeeded in obtaining latent infection in pigs (experiments with a strain of *Sp hispanicum* from O *maroccanus*) The experiments made at our request at the parasitological laboratory of the Sukhumi affiliated station of VIEM (All-Russian Institute of Experimental Medicine), involving infection of domestic animals with the spirochetes of tick borne relapsing fever (L Kuzmina) led to the disclosure of latent infection in a sheep and ass, whereas a calf proved resistant

In regard to cats, various data are available Ourselves and A Cheskis have observed only latent spirochetosis in kittens infected with the disease S P Kandelaki infected cats with the strain "Saganlug", while P P Popov and I Akhundov observed the death of kittens after inoculation with the Azerbaijan strain "Kerghez"

It may be concluded that domestic animals can hardly play any important part in maintaining and circulating the pathogen in question in the direct vicinity of man (economic biotopes), being secondary in this respect to commensate rodents However, the importance of domestic animals as massive alimentary hosts is great, which finds its confirmation in the frequently observed abundance of O *papillipes* in cattlesheds, where the sustenance of the entire mass of ticks cannot be provided by commensate rodents alone

ISOLATION OF SPIROCHETES FROM SUSPECTED PATIENTS

The basic diagnostic procedure is the discovery of spirochetes in the peripheral blood (thick film staining by the Romanowsky Giemsa method without preliminary alkalinisation by water)

In tick borne relapsing fever, spirochetes usually occur in small quantities, e g , 1 or 2 in several blood drops Their incidence is more frequent during attacks, but is sometimes revealed in interfebrile periods Cases are known when the spirochetes revealed at the onset of disease (on the 5th or 6th day) remained in the blood for 14 to 20 days In general no regularities were established in the appearance and disappearance of spirochetes in the peripheral blood while according to L Stavissky, 18 to 20 per cent of patients did not reveal spirochetes at all In rare cases healthy people have been found to carry spirochetes An important supplementary diagnostic method is infection of guinea pigs with the blood of patients applied to the nasal mucosa or conjunctivally, with subsequent dark field microscopy of stained blood films or fresh blood The procedure permits the isolation of spirochetes in patients whose blood seemed sterile at simple microscopy

For differential diagnosis it is necessary to remember that in some cases the disease may be accompanied by malaria which should accordingly be reflected in therapy Salvarsan treatment is generally acknowledged to be of little use in tick borne relapsing fever osarsol is not always reliable with mioarsenol relapses are observed in 10 per cent of all cases

Incubation lasts for a period from 4 to 7 days The onset is accompanied by chill temperature swiftly rising to 38 to 39° C, sometimes 40°, and general malaise The temperature curve is highly variable occasionally resembling that of malaria or brucellosis sometimes even irregular, which indicates the irregularity of attacks and different length of

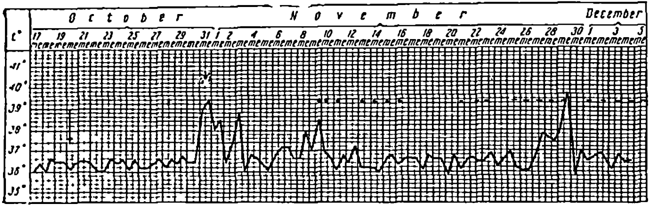

Fig 23. Temperature curve in tick-borne relapsing fever, provoked in a patient with paralytic dementia Crosses denote spirochetes in the blood

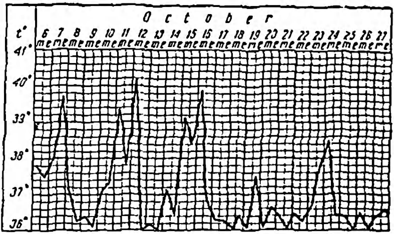

Fig 24 Temperature curve in spontaneous case of tick-borne relapsing fever, Northern Iran.

apyrexia. The number of attacks varies from 1 to 26 Total duration of disease—1 to 2 months.

When curing progressive paralysis by infection with tick-borne relapsing fever, the disease went on, without other therapy, for 5.5, 6, or even 7 months.

From time to time, the pathogen penetrates from the viscera to the peripheral blood. A laboratory case is known when spirochetes were revealed in the blood nine months after onset.

Differential diagnosis should envisage louse-borne relapsing fever and malaria.

EXPOSURE

Since contagion takes place during the suction of infected ticks, man is exposed to infection in places where he is liable to tick attack, viz, sufficiently large natural tick biotopes, e.g., caves, grottoes, natural ledges, artificial excavations (sumes in Turkmenia). Hungry ticks may also attack man in caves where their presence is inconspicuous. Cases of the kind have occurred during short visits to caves in the daytime. Often, infection takes place when people put up for the night in caves, grottoes and other places in the areas of distribution of vector ticks

In anthropurgic conditions, infection may take place on the premises of primitive buildings, in cattlesheds different abandoned structures and ruins within the geographic range of vector ticks which inhabit cracks in plaster and rat and mouse holes

Tick attacks are possible at any time of day and night, regardless of season provided that the ambient temperature does not arrest blood digestion or render the ticks immobile for long periods The immersion of the hypostome into the skin and subsequent suction are not felt by a sleeper, who does not wake up On repletion, the ticks crawl back to their biotope The site of tick bite develops a vivid skin reaction which testifies to the attack of *Ornithodorus* a circumstance of certain diagnostic and epidemiological importance

When collecting anamnestic data on a suspected patient, it is necessary to find out what places he visited during

Fig 25 Building of local materials—domestic focus of tick borne relapsing fever, Central Asia

the last 10 or 12 days and whether he had spent the night in nature or on premises likely to harbour ticks The disclosure of the typical skin reaction is an important index

The human population of vector inhabited areas, contract the disease at an early age Near Keraj (Iran) a case was recorded in a baby with all the typical manifestations—traces of tick bite on the skin and incidence of pathogens in the blood Repeated attacks of infected ticks on post-convalescents merely increase their resistance to renewed infection, whence, as a rule, fresh cases are mostly observed among people arriving from non endemic localities

Stable foci of the disease may form in separate buildings and even rooms As observed by Slavina (1944) in the settlement she visited, the disease was successively contracted by 9 newcomers residing on arrival in the same house

A similar observation was made in another locality, where the disease was successively developed by the inhabitants of a room in a primitive building, whereas no cases were noted among the neighbours This circumstance testifies to the unwillingness of ticks to migrate

Thanks to their high famine resistance, ticks may remain in abandoned buildings for long periods Hence, such premises should never be slept in A characteristic occasion was observed during one of our expeditions to Iran Cases were noted when people fell ill at a customs house located in a half ruined earthen building Prolonged investigations showed it to harbour infected ticks When the office was transferred to other tree sheltered premises further off along the dyke bank, the disease was no longer observed

SEASONAL PREVALENCE

According to the records of one of the hospitals in Tajikistan for 1923 1925, in the year 1925, peak incidence was observed in spring and the beginning of summer Monthly prevalence in relation to the overall rate was as follows from December to February—7 per cent, March May—58 per cent (May—36 5 per cent), June September—23 per cent, and October-November—9 per cent

Morbidity dynamics should be assayed among the newly arriving, non-immune section of the population, since the local inhabitants develop the disease at early ages, and hence prove unsusceptible to subsequent infection when occasionally exposed to the attacks of infected vectors In the town of Osh, Southern Kirghizia, peak morbidity was observed in August 1937, and in May July 1935 (Y N Pavlovsky, A Alymov, 1939)

Sporadic cases may likewise occur during the cold season, since O *papillipes*, residing in primitive dwellings and services, may retain its mobility throughout the year

In experimental conditions, this tick may feed and transmit spirochetes even at +5° C (Y N Pavlovsky and A N Skrinnik) In the Pamirs, O *papillipes* is revealed at an altitude of 2800 m above sea level (G Y Zmeyev)

Occasionally, unexpected outbreaks of tick borne relapsing fever are observed Thus, in one of the towns in Tajikistan, long known to be focus of the disease, approximately 200 cases were registered in 1940 1941 The local cottages were constructed on the European pattern, the walls and other parts however, being taken from older structures to accelerate erection Earlier, it had been established that wall fissures in the old huts contained infected vectors Many newcomers were accommodated in such cottages which was the cause of the observed morbidity

PROPHYLAXIS

Prophylactic measures against tick borne relapsing fever are adopted for two purposes protection of man against attacks of vectors and anti tick treatment of the environments—domiciles, services, and the natural biotopes of ticks (rodent burrows caves, grottoes, etc) The surest way of preventing infection is to avoid sleeping in primitive shelters which may be suspicious in regard to infestment with vector ticks This, obviously, concerns localities within the vectors' range of distribution In any case, it is better to spend the night in the open air—in yards, gardens, or at *any rate, within a fair distance from earthen walls*

A question still unclarified is whether *Ornithodorus* abandon their burrows for feeding only at night or at other times too Investigations to this effect should be made locally and by local means and personnel Various safeguards have been proposed for protection of sleepers against tick bite, the most reliable being the use of bed nets suspended around and above the bed The bedstead should be placed at a distance from the walls, and the legs put in bowls or cups of water This, however, is not always practicable Tar emulsion and mint tincture have been used as repellents, but their effects are too short lived

In their natural biotopes, such as caves, hungry ticks may attack man in the daytime Cases are known when tick borne relapsing fever was contracted after short visits to half blocked caves, where ticks had not been detected Individuals collecting vector ticks in nature fall ill almost invariably Protective suits in such cases are unusable, due to unreliability and impossibility of long wear owing to intolerable heat The urgent need for developing adequate repellents is evident

As regards sterilisation of dwellings, a useful measure is general repair, plastering of walls, especially fissures and panelling, reliable blocking of rat holes which may contain spirochete carrying rats and vector ticks

The use of chlorine and chloropicrin for eliminating ticks in earthen buildings is technically difficult The inner surfaces of a fumigated domicile continue to exude gas for long periods, which renders it uninhabitable

Many authors, including our own colleagues, have tested preparations of DDT and hexachlorane, but within their habitats in human dwellings, *Ornithodorus* ticks are almost inaccessible to the effects of contact poisons, and, besides, are resistant to the said preparations The latter are used in the form of emulsions and suspensions for spraying or as powders for dusting According to I Teravsky and A Shustrov, the resistance of ticks in different metacyclic stages may vary, being higher in the III, IV and V nymphal stages in males and females On contact with poison, larvae and nymphs of the I and II stages lose mobility in several hours, dying after 5 to 20 days Nymphs of greater age, males and females, may survive in 10 25 per cent of all cases

Tick infested premises should be treated with DDT and hexachlorane preparations with due regard for the existing rules for handling the respective substances

Special thoroughness is necessary in treating the bottom of walls, cracks, niches, fissures, holes, concentrations of refuse and loess on floors and in corners liable to harbour ticks Repeated treatment should preferably follow after one or one and a half months Three or five days after treatment, all cracks should be plastered holes blocked and refuse removed The advisable time is summer and autumn when the larvae have hatched and the first nymphs appear, the latter being less resistant to the preparations, which, however, does not rule out control measures at other seasons

The approximate dosage of DDT and hexachlorane should be 3 gr ADV per sq m of surface The dose is calculated according to the strength of the technical preparation contained in the dust or emulsion The natural biotopes of the ticks—burrows, caves, grottoes—are treated with hexachlorane, which is taken in the ratio of 4 to 6 gr ADV per hedgehog or turtle burrow and 3 gr per sq m of surface in caves

Hexachlorane has a swifter toxic effect on ticks than DDT, and therefore should be used in farm and service buildings and natural biotopes of ticks It is likewise advisable to treat tick-infested dwellings with hexachlorane, provided they are thoroughly aired before receiving inhabitants. The most effective way to eliminate foci of tick-borne relapsing fever in settlements is to construct buildings in conformity with sanitary requirements, to plant parks on waste areas, and destroy rodents and their burrows. Control of the foci will be greatly enhanced by extensive economic development of wasteland, wide-scale hexachlorane treatment of the natural biotopes of ticks (burrows of wild animals, caves, etc.) and extermination of rodents.

Occasionally, burrows of wild animals may be found in ravines, road ditches, half-destroyed earthen walls, and other places on cultivated land where people stop to rest. Elimination of such foci is effected by hexachlorane treatment with subsequent checking of its results. If necessary, treatment may be repeated. On large areas, where broad-scale control campaigns are carried out against rodents as general reservoirs of acute infections (as in some of the republics of Central Asia and Transcaucasia), the work should be done in accordance with the mentioned requirements, envisaging the elimination of foci of tick-borne relapsing fever as well as similar diseases with natural foci (cutaneous leishmaniasis, etc.).

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LEPTOSPIROSES

HISTORY OF RESEARCH

The leptospiroses were first defined as an independent class of diseases in the second half of the nineteenth century In 1886 Weil described a peculiar febrile condition accompanied by hypertrophy of the spleen, jaundice and inflammation of the kidneys, which he distinguished from other forms of icteric diseases Outside *the U S S R this* clinical form is known as Weil's disease or icterohemorrhagic leptospirosis N P Vasilyev (1888) presented more convincing data for the recognition of this disease as an infection *sui generis*, which he named infectious jaundice N P Vasilyev investigated 11 cases of the disease from 1883 to 1888 and analysed 37 cases described up to 1888 in other countries By his definition, the disease presents an acute infectious form with high temperature and in most cases symptoms of predominant lesions of the central *nervous system, liver and kidneys*

N P. Vasilyev collected data on the seasonal and professional prevalence of the disease, which permitted it to be distinguished from other icteric diseases, including epidemic hepatitis, then known as catarrhal jaundice Hence, in literature, this clinical form, which was first described by N. P Vasilyev and Weil and presents the primary clinical type of leptospirosis, is justly named Vasilyev-Weil's disease (synonym icterohemorrhagic leptospirosis)

The hypothesis concerning the infectious nature of Vasilyev Weil's disease was confirmed 26 years later when the Japanese authors Inada and Ido (1914) discovered its causative agent, which they gave the name of *Spirochaeta icterohaemorrhagiae* [synonyms *Sp icterogenes* (Uhlenhuth and Fromme), *Sp nodosa* (Hubner and Reiter)] Later, Noguchi, issuing from the biological and morphological similarity between *Spirochaeta icterohaemorrhagiae* and saprophyte spirochetes [*Sp biflexa* (Wolbach, Binger, 1913)], classified them as an independent genus—Leptospira (leptos—thin, speira—spiral, Noguchi, 1918).

CAUSATIVE AGENT

In preparations fixed with vapours of osmic acid and stained by the Romanowsky Giemsa method, leptospires assume a reddish violet colour Their length is 7 to 14 μ, seldom up to 30 40μ thickness—0 25 0 3μ The amplitude of the primary coil is 0 45 0 5 μ, height—0 3 μ; one or both ends are hooked

With the advent of electronic microscopy, the morphological description of leptospires was somewhat modified (Morton and Anderson, 1943, Babudieri, 1958, et al) The average length of leptospires is now known to be 4 to 18 μ, diameter—0 07 to 0 14 μ, primary coil amplitude—0 25 μ and height—0 3 μ Flagellate structures, granules, vacuoles or other inclusions have not been revealed The body comprises a central axial thread (approx 0 02μ in dia) and a cytoplasmic spiral evenly surrounding the former The cytoplasm is covered with a delicate structureless membrane The axial thread is believed to be a skeletal structure and organ of mobility Being secured to the internal membrane and contracting at regular intervals, it causes the spiral to rotate, thus effecting its locomotion through liquid and semi-liquid media (Babudieri, 1958)

The identification and study of leptospires in general biological practice is based almost exclusively on microscopy of preparations of live leptospires (by the crushed or hanging drop techniques) The organisms refract light with difficulty, due to which they are examined by dark-field microscopy (with a paraboloid or cardioid condenser, etc), the object being side illumined and slightly magnified (e g , with a 40x object glass and 10x eyepiece) In dark-field examination they appear as silvery white threads, consisting, as it were, of equal sized granules or regular discs closely adhering to one another (primary spiral coils), one or both of their ends terminating in hooks, although hookless forms are met as well At the ends there are also button like swellings (granules) The hooks present a continuation of the plasmic spiral Survival forms (spores, etc) have not been discovered

An essential diagnostic feature of leptospires is their mobility, which changes according to the density of the medium In water and liquid nutritive media they perform (a) rotary movements on one spot, caused by simultaneous rotation of both terminal hooks, the median part (body) of *the leptospire being drawn out in a straight line,* (b) rectilinear progressive motion in the direction opposite to the rotation of the hook (c) circular motion In semi liquid media (e g , 0 1 to 0 2 per cent agar +10 per cent rabbit serum) leptospires move sinuously, the body twisting into waves and losing the shape assumed in liquid media

Leptospires are typical hydrobionts, i e , they are able to live only in media containing water in quantities sufficient for locomotion The saprophyte types inhabit the water in natural and artificial reservoirs, while pathogenic leptospires are contained in the blood lymph, primary urine of the kidney channels and the spinal fluid of man and animals Drying causes rapid death The microbes are non-resistant to light, high temperature and variations of medium pH below and above the optimum pH=7 2 7 4 Reproduction is effected by transverse fission

Besides the causative agent of icterohemorrhagic leptospirosis (Vasilyev-Weil s disease) approximately 40 serotypes have been revealed, all pathogens of human leptospiroses These organisms have been named by the binary nomenclature (e g , *Leptospira batavia, L canicola*, etc), i e , they are each regarded as a separate species

The specific classification of leptospires is based on serologic (antigen) distinctions revealed in the reactions of agglutination and lysis The Rieckenberg test is not sufficiently specific, and complement-fixation too involved The serotypes are markedly specific, having a stable antigen structure (Table 1)

Table 1

Species (Serotypes) of Pathogenic Leptospires and Leptospiroses Occurring in the U S S R

Species (serotype) with synonyms	Clinical form	Epidemiologic features and reservoirs
L icterohaemorrhagiae (Inada a Ido 1914) *L icterogenes L Nodo a* type A	Vasilyev Weil's disease icterohaemorrhagic leptospirosis group of icteric leptospiroses Lethality up to 10 per cent	Occurs more often in urban than *in rural* localities in presence of principal reservoir—grey rats Secondary reservoir—dogs Infection *transmitted through* water (during bathing drainage sewerage and other work) food furniture contaminated by the urine *of rats* Seasonal prevalence—summer and autumn
L grippotyphosa (Tarasov 1928) *L itullina* type I	Water fever group of non icteric leptospiroses Lethality negligible	The most widespread form of *leptospirosis* in the U S S R Occurs more often in urban than in rural localities Reservoirs—rodents *Cricetulinae Muridae* (root vole common vole field vole field mice etc) and cattle Man infected when hay *making on* swampy fields bathing in small boggy ponds tending sick cows or drinking their milk

(Continued)

Species (serotype) with synonyms	Clinical form	Epidemiologic features and reservoirs
L pomona Klayton & Derrick 1936 FFC Monyakov *type II*	Pomona leptospirosis group of non icteric leptospiroses	Small isolated outbreaks and separate cases associated with bathing in ponds contaminated by excreta of eliminator pigs and tending of the latter (Schweinhuterkrankheit) Cattle less important in dissemination *Reservoirs in nature*—field and domestic mice grey rats
L tarassovi L FFA (Tarasov 1938) type III *L hyos L mitis* (Johnson)	Far Eastern leptospirosis non icteric group	Rarely occurs in the Soviet Union (Far East Altai territory) Reservoir—cattle Natural reservoirs unknown
L canicola (Klarenbeeck Schuffner 1932) type IV	Canicola fever Cases with jaundice recorded in the U S A and *Denmark In the* U S S R only *non icteric cases* are known	Occurs in individuals maintaining close contact with reservoirs owners of sledge dogs and domestic pets *veterinarians dog pound* personnel etc Grey rats *suspected as reservoirs* (Sakhalin)
L bataviae (Walch 1926)	Batavia fever Non icteric forms recorded in the U S S R and Italy In Indonesia *assumes severe* form with jaundice	Sporadic cases in Byelorussia Main reservoir—harvest mice (Yaroslavl region Byelorussia Altai territory Northern Kazakh*stan)*
L nero (Ananyin and Karasyova 1950) *L Saxkoebing*	Nero leptospirosis Non icteric	Cases occur during haymaking on swampy floodland around lakes and rivers (Yaroslavl and Smolensk regions *Primorye territory*) Reservoirs—root voles *and eastern vole*
L hebdomadis Ido Ito Wani 1916 *L Akiyami B*	Seven day fever Non icteric	Sporadic cases in Primorye territory associated with bathing in small ponds Reservoir—eastern vole
L kasachstanica I Krepkogorskaya 1948	Kazakhstan fever Non icteric	Southern Kazakhstan Reservoir—large and Tamarisk gerbils

(Continued)

Species (serotype) with synonyms	Clinical form	Epidemiologic features and reservoirs
L kazachstanica II Krepkogorskaja 1948	Ditto	Ditto
L ussuri Kraminskaja and Eshin 195_	Non icteric *Hebdomadis gr*	Primorje territory Reservoirs in nature not definitely known
L erinacei auriti Ananyin 1949	Not definitely established	Sporadic cases Infection known to be widespread in Stavropol territory among long eared hedgehogs
L poi Mino 1941 *L sorex*	Ditto	Sporadic cases in Western Europe *Leptospires* revealed in the kidneys of common shrews root voles and common voles
L erinacei europaei Ananyin 1951	No cases recorded in man	Infection discovered in European hedgehogs (Moscow region)

The pathogenicity of different species may vary widely However, the clinical picture, in man as in animals, though caused by different leptospires, is largely stereotype, owing to similar pathogenesis in all leptospiroses, which is based upon a lesion of the nervous system and functional disturbances in other tissues and organs, especially the liver and kidneys

CLINICAL FEATURES

Generally speaking, none of the symptoms are associated with any one species or subspecies of leptospires It is possible to speak only of the prevalence of certain symptoms in cases brought about by a given leptospire (icteric, non icteric and meningeal forms) and greater or lesser severity (malignant cases with high lethality and benign forms with a negligible death rate), associated with the pathogenicity of the given species of the causative agent

In malignant forms, symptoms of a severe lesion of the nervous system are accompanied by marked afflictions of the liver and kidneys One of the most vivid manifestations of liver pathology is jaundice of the skin and mucous membranes

The clinical forms of leptospiroses are divided into two groups icteric (malignant), in which jaundice occurs in 50 and more per cent of all cases, with up to 10 per cent lethality, and non icteric (benign), in which jaundice and lethality are extremely rare In the Soviet Union only one icteric form is known to occur, viz, Vasilyev Weil s disease The group of non icteric leptospiroses includes water fever (causative agent *L grippo typhosa*) and leptospiroses caused by *L pomona, L tarassowi* (syn *L mitis*), *L canicola, L nero* (*L saxkoebing?*), *L kasachstanica* I, *L kasachstanica* II

Experiments on guinea pigs weighing 100 to 200 grams and other laboratory animals, provided ample data justifying the division of leptospiroses into two groups In guinea pigs infected with *L icterohaemorrhagiae*, the icterohemorrhagic leptospirosis proceeds with high temperature, jaundice, hemorrhages in the organs, and almost invariably has a lethal outcome Guinea pigs infected with *L grippo typhosa* or other species of the non icteric type, have mild forms of the disease with brief (2 or 3 days) temperature rises and inhibited growth, the outcome is mostly favourable

EPIDEMIOLOGY

Epidemiologically, the leptospiroses differ essentially from other zoonoses with natural foci in that no blood sucking vectors of the pathogen have as yet been definitely established in the natural and anthropurgic foci of the disease Transmission through water is proved beyond all doubt

The epidemiologic importance of man as a reservoir is null, although post convalescents may occasionally be eliminators, which is explained by certain properties of the leptospires, pathogenetic features of the infection and circumstances of social nature The leptospires remain in the convoluted tubules (I and II orders) of the kidneys and are eliminated with the urine The amount of leptospires in such urine is usually negligible, their viability mostly low, whence in acid urine they swiftly perish Infection through contact with objects surrounding the patient and contaminated by the latter's urine is almost impossible due to the pathogen's low resistance to desiccation Epidemiological instances of infection by such routes have not been recorded

The reservoirs of pathogenic leptospires are many species of rodents, *Insectivora, Artiodactyla, Perissodactyla, Carnivora, Cheiroptera* (Table 2)

The importance of these reservoirs in disseminating infection is unequal, but the mechanism of transmission is similar in all

Both animal hosts and human post convalescents maintain leptospires in their kidneys (convoluted tubules of the I and II orders) and eliminate them into the environment with their urine Man may contract the disease while bathing or at work (drainage, bridge construction, etc), and when drinking water or washing in reservoirs contaminated with the urine of pathogen carriers, etc Less important is infection through food, furniture, domestic utensils, etc, contaminated with animal urine Cases are known of infection contracted while tending diseased agricultural animals and dogs or when trapping leptospire carrying rodents Contagion takes place at contact with animal urine containing leptospires The portals of entry of the infection are damaged skin and mucosa (of the eyes, gastro-intestinal tract, etc)

The principal reservoirs of leptospires pathogenic for man are certain species of rodents (chiefly, *Muridae* and

Cricetulinae), which may be either synanthropous or inhabiting in nature, where the infection usually assumes a chronic course After infection, which takes place through damaged skin and mucosa, the leptospires circulate in the blood for 5-10 days, pending which they concentrate in the convoluted tubules of the kidneys, from whence they are discharged with the urine Pathogens may continue to be revealed from several days to a year (findings of experiments on common voles) The amount of leptospires in the urine is highest during the first four weeks after infection (often even higher than in cultures) Initially, the host develops interstitial nephritis Chronic carriers reveal sclerotic changes in the kidneys

In natural conditions, rodents do not die of leptospirosis but their activity is somewhat reduced Infected animals become the victims of predatory birds more frequently than healthy ones

Table 2

Animal Reservoirs of *Leptospirae* in the U S S R
(including animals attested as hosts by isolation and identification of pure leptospire cultures)

Animal host	Leptospire species	Features of disease in animals epidemiological importance
Cattle	*L grippo typhosa* *L pomona L tarassovi*	Normally assumes severe course with high lethality Widespread in southern and eastern regions Convalescent animals may be source of infection for man
Pigs	*L pomona*	Disease mostly latent occurs on farms mostly due to violations of sanitary and veterinary rules Reservoir animals are source of human infection
Dogs	*L canicola*	Course acute or chronic Cases of human infection described in Primorye territory and Sakhalin

(Continued)

Animal host	Leptospire species	Features of disease in animals, epidemiological importance
Silver fox	*L. pomona*	Observed on fox farms, course acute with high lethality. Epidemiologic importance not established
Horses	*L. grippotyphosa* *L. pomona*	Course either acute (10-60 per cent of cases lethal) or latent. Cases of human infection from animals unknown
Common shrew (*Sorex araneus*)	*L. pom., L. grippotyphosa*	Incidence established in central regions, Altai and Stavropol territories, Kazakhstan. Cases of human infection unknown
European hedgehog (*Erinaceus europaeus*)	*L. erinacei europaei*, *L. sorex*	Carriers discovered in Moscow and Yaroslavl regions. Epidemiologic importance not clear
Long-eared hedgehog (*Erinaceus auritus*)	*L. erinacei auriti*	Considerable incidence of infection in Stavropol territory where human cases are also on record
Black polecat (*Putorius putorius*)	*L. grippotyphosa*	Leptospires isolated from kidneys of polecat caught in a natural focus of leptospirosis (Yaroslavl region)
Grey rat (*Rattus norvegicus*)	*L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*	More or less high incidence established in Moscow, Kiev, Leningrad and other cities. Main source of icterohaemorrhagic leptospirosis. On Sakhalin and in one of the central regions rats have been found to carry *L. canicola*
Bank vole (*Clethrionomys glareolus*)	*L. grippotyphosa*	Relatively low incidence recorded in central and western regions
Field mouse (*Apodemus agrarius*)	*L. grippotyphosa* *L. pomona*	Leptospire carriers revealed in Kalinin and Yaroslavl regions, Stavropol territory, Leningrad region, Byelorussia

(*Continued*)

Animal host	Leptospire species	Features of disease in animals epidemiological importance
Root vole (*Microtus oeconomus*)	*L grippo typhosa*, *L nero* (*saxkoebing*) *L sorex*	One of the chief reservoirs of infection in the central regions (Moscow, Yaroslavl, Smolensk), Altai territory and North Kazakhstan
Common vole (*Microtus arvalis*)	*L grippo typhosa*, *L pomona L bataviae*	Along with other voles, principal reservoir of infection in individual regions of European and Asian USSR
Bush vole (*Microtus majori*)	*L pomona*	Leptospire strains isolated from animals in Stavropol territory
Water rat (*Arvicola terrestris*)	*L grippo typhosa*	Infected animals revealed in central regions and Byelorussia
Forest mouse (*Apodemus silvaticus*)	*L grippo typhosa*	Infected animals found in central regions, Stavropol territory and Byelorussia
Harvest mouse (*Micromys minutus*)	*L bataviae*	Carriers found in Yaroslavl region, Altai territory, North Kazakhstan, Byelorussia Human infection recorded in Byelorussia
Eastern vole (*Microtus fortis pelliceus*)	*L nero* (*saxkoebing*), *L hebdomadis* *L grippo typhosa*	Reservoirs found only in Primorye territory
Domestic mouse (*Mus musculus*)	*L pomona*, leptospires of the *Hebdomadis* group	*L pomona* revealed in Leningrad region, *L hebdomadis* —in Akmolinsk reg , Stavropol territory
Common field vole (*Microtus agrestis*)	*L grippo typhosa*	*Infected animals found in* Gorno Altaisk autonomous *region and Moscow region*
Narrow skulled vole (*Stenocranius gregalis*)	*L grippo typhosa*	Reservoirs in Altai foothills
Common hamster (*Cricetus cricetus*)	*L grippo typhosa*	Carriers found among hamsters in the same region
Large gerbil (*Rhombomys opimus*)	*L kasachstanica* I, *L kasachstanica* II	Regarded as a potential reservoir in Southern Kazakhstan
Tamarisc gerbil (*Meriones tamariscinus*)	Ditto	Ditto

Each serotype of the leptospires pathogenic to man is linked with a definite group of animal hosts, the importance of different species of which in maintaining and disseminating the disease is unequal

According to our own findings and available literature, the hosts of *Leptospira grippo typhosa* in the central regions of the Soviet Union may be root voles, water rats, common voles, bank voles, forest mice and field mice Among these, as regards incidence of infection, number and ecologic proper ties conducive to the infection of man, root voles, common voles and water rats stand foremost The reservoirs of the pathogen of so called Nero leptospirosis (*Hebdomadis* group) are root voles and reed voles Harvest mice are the reservoir of *L batavia* As commonly known, *this species of rodents is the reservoir of L batavia* in other European countries as well Grey rats are a reservoir of icterohemorrhagic leptospires on all five continents

Depending on the habitat distribution of species of *Muridae* acting as reservoirs of individual types of pathogenic leptospires, the respective leptospiroses may be more or less permanently associated either with wild nature or territories inhabited and cultivated by man The specific natural characteristics of the foci of water fever—the leptospirosis most widespread in the Soviet Union—are conditioned by the fact that the reservoirs of *Leptospira grippo typhosa*—the pathogen of this disease—are root voles, water rats, forest mice and bank voles, which inhabit uncultivated or undercultivated places like swamps, floodlands and forests

Grey or common voles generally inhabit cultivated areas (sown fields, meadows) *Their ecology and biology stand in* direct relationship with the economic activity of man The foci of water fever, in whose formation these reservoirs of *L grippo typhosa* act the principal part, bear all signs of anthropogenic influence (e g, harvest-time outbreaks of water fever in Germany)

Grey rats, hosts of icterohaemorrhagic leptospires, live both in nature and, more preferably, in human domiciles and services Hence, cases of infection with Vasilyev-Weil's disease are more often registered in settlements than in nature The penetration and reproduction of harvest mice lies at the root of Batavia-type outbreaks among rice workers in Italy.

The maintenance of natural foci of leptospirosis is ensured by the continuity of the epizootic process among *Muridae* Such continuity, being based on the chronic character of the disease in rodents, which promotes the interseasonal maintenance of pathogenic leptospires in the rodent population, is dependent likewise on the routes of *pathogen transmission associated with the biology* and ecology of the reservoir and environmental factors of climate, geography, terrain and meteorology

The basic routes of transmission are through contact and water *Of somewhat lesser importance is infection through* food The portal of entry may be damaged skin and oral, nasal, conjunctival or genital mucosa Certain importance should be attributed to copulation, since in natural environments, this form of contact is most common during periods of rodent population growth, and the route of the pathogen's passage from animal to animal shortest

Intrauterine infection of progeny by parent rodents has not been revealed and is of no practical importance Likewise unestablished is the participation of arthropods—specific and non specific rodent parasites—in the circulation of infection in natural foci

In the central zone of the European U S S R the interseasonal epizootic dynamics of leptospirosis among root voles are irregular According to observations of voles in a floodland focus near lake Nero (V V Ananyin and E V Karasyova, 1950-1954) these dynamics, in their relation to the conditions of transmission, appear as follows

In the winter months the incidence of the infection among animals stays at minimum, since transmission through water is null, contact and food transmission being restricted (lower populations, almost complete stoppage of reproduction). Early in spring, due to flood waters and concentration in survival habitats, as well as the onset of mating, direct and indirect contact increases along with the incidence of leptospirosis. As the waters recede and the animals disperse among their natural habitats, copulation, and hence, sexual contact, become more frequent. The reproduction of the animals provides for the appearance of more and more generations susceptible to infection, with the resultant increase in density of population. In summer and early autumn (July-September) the population of rodents as well as direct and indirect contact between them, is at its height and the rate of leptospire infection among voles, therefore, is greatest. Abundant rains enhance infection through water, moist soil, and green food contaminated with the urine of pathogen-carriers. Late in autumn (October-November) the reproduction rate diminishes together with the population, which is followed by an increase in the number of post-convalescents unsusceptible to repeated infection. The epizootic approaches its minimum. A small percentage of the animals infected during spring and summer survives the entire winter, still remaining leptospire-carriers. In spring these animals are liable to cause a new uprise in the epizootic.

The boundaries of a focus are determined by topographic conditions conducive to the life of infection-carrying rodents.

The organisation of preventive sanitation in the natural foci of leptospirosis, as in other foci, requires an assay of the latter's structure. With this view, the following factors are estimated: (1) nomenclature of primary and secondary hosts among wild and domestic animals; (2) dynamics of the epizootic process among the principal host populations (in relation to habitat distribution, age and sex of animals, meteorologic and other factors), (3) seasonal changes in the *territorial location of natural foci*

Infection carriers are revealed in the foci with the aid of epidemiological data, viz, an approximate estimate is taken of the sites of human infection (e g, boggy hayfields, *bathing-pools, drainage and harvesting areas, etc*), making sample surveys of the local rodents for leptospire infection In absence of epidemiologic indications a fairly good landmark are certain habitats populated by small rodents, *which have frequently been found to harbour infection in* earlier investigated foci, such habitats including damp, swampy areas of river floodlands, lake shores, hollows, etc For example, animals infected with *L grippo typhosa* and other pathogens of non icteric leptospiroses, were revealed in swamps and along the banks of lakes, rivers, old riverbeds, etc, in already explored foci of the Moscow, Yaroslavl, Smolensk and Akmolinsk regions, Altai territory and elsewhere The animals included representatives of the genus *Microtus*, harvest mice (*Micromys minutus*) bank voles (*Clethrionomys glareolus*), water rats (*Arvicola terrestris*) *In the Yaroslavl region root voles (Microtus oeconomus)* infected with leptospires were caught only in reed thickets, among grass grown hummocks, in wet scrub and other habitats of a lake basin (V V Ananyin and E V Karasyova, 1953) In Northern Kazakhstan infected root voles and domestic mice (*Mus musculus*) were caught among the reeds near fresh water steppeland lakes (*V V Ananyin and E V Karasyova, 1956) In the Primorye* territory infected reed voles were met in hummocky swamps (V A Eskin, N N Isofatenko, 1952, et al)

Certain importance may be ascribed to the alkalinity and salt content of the water As is known, leptospires can not survive in water with a high salt content For this reason, no natural foci of leptospiroses have been discovered *on sphagnum and peat bogs (where the water has an acid* reaction), or along the banks of saline reservoirs which lack the conditions necessary for transmission through water

The animals are caught in order to assay the incidence of infection, population numbers and habitat distribution, which is indispensable for a complete appraisal of the focus in question.

Population counts may be either relative or absolute. The former are carried out by means of spring-traps, wire cages and ditches. Absolute counts may be effected by marking the animals.

Best suited for the purpose are medium-sized spring-traps and wire cage-traps, the cage in which may be made of a common preserve can. The cage is intended to protect the trapped animals from bad weather and hunger until they are extracted, for which the cage is provided with a supply of food and straw lining. The traps are baited with combination bait consisting of bits of rye bread crust dipped into sunflower oil and slices of carrot. Such bait will prove attractive both for grass-eating voles and seed-eating mice.

The traps are set in rows of 100 with 7 metre intervals between traps. The rows are distributed as regularly as possible among all habitats under survey, moist areas being given priority. The traps are set in the afternoon, and checked and removed early in the morning (in the temperate zone, not later than 6-7 a.m. during the warm season). The mean number of animals caught in a night per 100 traps (trapping percentage), is assumed as an index of the relative population of small mammals. This method affords an estimate of animal population changes in time and the simultaneous level of animal population in different habitats.

Traps, even combination baited, are not equally effective for different species. For example, shrews, birch-mice and certain other species are seldom caught by this method. Therefore, to achieve the greatest possible accuracy in counting the small mammals populating the foci, use is made of ditches. The latter are dug spade-deep, spade-wide, and 20 m long, with smooth sides. Each should be furnished with 3 tin cylinders 60 cm high and 30 to 35 cm in dia which are dug into the bottom at equal distances (8 to 10 m) from one another The upper rim of the cylinder should closely fit between the walls of the ditch The ditches are inspected daily, not later than 6 or 7 a m The mean number of animals caught in 10 days per ditch presents a relative index of the population of a given habitat, as counted by the method described The ditches require regular care, i e, they should be systematically cleared of accidentally occurring insects, frogs, etc, and the walls prevented from crumbling, and so on

Absolute counts provide more accurate data, permitting estimates of the number of animals per unit of area In the natural foci of leptospirosis, where the infected animals live on damp, swampy terrain and thereby render counts by means of wholesale trapping (excavation of burrows) impracticable, the animal population is counted by marking The procedure involved is as follows Wire cage traps are staggered over an area of 1 or 2 hectares with 10 metre intervals between traps The devices are inspected twice a day Captured animals are marked by cutting off their toes in different combinations (digital numeration) and set free at the site of capture Subsequent repeated catches of marked animals make it possible to determine the number of permanent animal inhabitants in the area under survey, i e, the absolute animal population

The dead and living animals obtained for laboratory study are delivered separately All specimens extracted from spring traps, ditches, etc, are placed into special bags, one per bag, accompanied by labels giving date, brief description of habitat, place of capture and name of collector Live animals caught in snares are placed in special cages with separate numbered cells provided with doors Each animal is put into a separate cell The labels are filled in the same manner as for dead animals, except for the number of the respective cell

In dead animals apart from specific identification, the zoological investigations conducted prior to autopsy and bacteriological study should include measurements of the length of the body from the tip of the snout to the root of the tail with an accuracy of 1 mm, and weighing (accuracy up to 1 gr), which is necessary for determining the rodent's age. After autopsy and bacteriological tests the condition of the genital organs is surveyed to determine the reproductive activity of the given specimens. In males, the length of testis and seminal vesicles is measured to determine the occurrence or non-occurrence of spermatogenesis. In doubtful cases microscopy of smears obtained from seminal adnexa (for spermatogenesis) should be resorted to. In females, maturity is established by the status of the uterus and ovaries.

In pregnant females, an estimate is made of the number and size of the embryos, and in those who have had offspring—the occurrence of lactation, counting the number of placentary spots on the uterus (an index of the number of previous litters and littered young).

For more precise age estimates, skull series are collected. Assessments of age and reproductive activity are important for drawing correct conclusions on the conditions of the development of the epizootic process among the rodent population. As is known, leptospirosis chiefly affects mature animals, the possibility of infection increasing with age. In our own investigations conducted in the Yaroslavl region, there were no infected specimens among young root voles up to 1.5 months old, miscellaneous cases occurring at 1.5-2 months, and more than half the voles from 2.5 to 4 months old being infected, while as regards animals over 8 months old, the absolute majority were involved in the epizootic.

The age composition of an animal population varies annually. For some years in autumn it may include a considerable percentage of rodents surviving from the previous winter, while for others the entire population may consist of animals borne the same year. In the former case, accordingly, the rate of the epizootic among the rodent population is higher than in the latter.

By confronting the age composition and rate of reproduction with data on number and habitat distribution, a long-term population forecast may be made, e g , in spring for autumn, and in autumn for the spring of the next year, which is obviously important for the organisation of anti-epizootic and anti-epidemic campaigns, since the morbidity rate of an epizootic of leptospirosis depends on the density of the given rodent population The higher the latter, the greater the possibility of healthy specimens being directly infected by eliminators of leptospires (through copulation, residence in common burrows, etc), and through food and water contaminated by urine of infected animals

Apart from number and age composition, the rate of an epizootic among a rodent population depends on the amount of sediments, greater sediments in summer mostly being accompanied by higher morbidity, since water, as is known, presents one of the principal routes of transmission By confronting these three factors, a more or less accurate forecast may be made of the rate of the epizootic for the forthcoming season or year, which to a certain extent permits epidemiologic prognosis as well

TECHNIQUE OF INVESTIGATIONS FOR LEPTOSPIROSIS INFECTION IN WILD ANIMALS

Among the methods employed are (1) dark-field microscopy of saline suspensions of crushed organic tissues (kidneys, liver) and urine, (2) microscopy of histologically prepared kidney and liver sections (silver impregnation), prior to histological treatment, the preparations are kept in 10 per cent formalin, (3) inoculation of material under test into special culture media (Vervoort-Wolf's, Fletcher's, etc), during expeditions, when working in impromptu laboratories, it is important to have a store of media contained not in conventional bacteriological test tubes, but in ampules which are opened before and soldered after inoculation; (4) infection of laboratory animals with suspensions of the organs of animals under study; (5) serologic (agglutination-lysis) tests for antibodies active against pathogenic leptospires.

The above methods have been attested in application to studies of leptospire infection in wild rats, the literature on the subject being sufficient for assaying the advantages and shortcomings of each.

Some of the technical details of investigations for leptospire infection employed by ourselves require special consideration, as somewhat differing from the routine.

The carcasses of animals (those caught alive should be killed) are secured on the autopsy table. The pelt is treated with alcohol and seared (we, personally, do not resort to disinfectants like lysol for fear of accidental contamination of investigated material). The instruments for autopsy (scissors, tweezers, etc.) are placed before use into 96° alcohol and burned in the flame of a spirit-burner directly prior to operation. The pelt on the abdomen and thorax is separated and folded aside.

With sterile instruments, an opening is made first in the abdominal, then thoracic cavities, removing the liver and kidneys and placing them in sterile cups. With the aid of a Pasteur pipette, 2 or 4 drops of blood are taken from the heart, each drop being separately applied to a piece of filter-paper and conserved by 2 or 3 hours drying at room temperature for use in serologic tests. In cases when the pipette is of no avail, we resort to dissection of the cardiac cavity.

In contrast to certain authors (A. A. Varfolomeyeva, V. I. Terskikh), who recommend preliminary crushing of material for primary culture and microscopy in porcelain cups, we find this procedure not only too complex for mass investigations on rodents, but also quite unsatisfactory for a number of reasons. (1) Crushing may often lead to contamination with alien microflora. (2) Due to the presence of leptospires in the organ and the respective antibodies in the blood, crushing leads to swift contact between the aforesaid, which, as we have often observed, unfavourably tells on the results of microscopy and culturing, owing to the accompanying agglutination lysis reaction (3) Within the kidneys, as is known, the leptospires disseminate in the upper, cortical layer, whence culturing and microscopy of tissue suspensions made of the entire organ are unadvisable With this in mind, we prepare cultures not from suspensions, but from pieces of tissue cut out by scissors, with due regard for sterility, from the surface of the kidney, i e , the cortex The tissue samples thus obtained may then be extracted and inoculated with Pasteur pipettes

The volume of tissue employed for culture should approximate a millet grain, since, as we have found, large quantities of culturing material are not only unconducive to the growth of leptospires, but even hinder it owing to subsequent maceration of the organic tissues and changes in the mechanical and chemical properties of the media For dark field microscopy, a so called crushed drop preparation is made on a slide from a saline suspension of the organ The material for suspension is extracted from the cortex with a Pasteur pipette Whenever the bladder is filled with urine, the latter is also taken for culture and microscopy In cases when direct microscopy reveals leptospires in an animal's kidneys, it is advisable to infect guinea pigs (subcutaneously) with a suspension of kidney tissue to estimate the pathogenicity and virulence of the microbes

Leptospire strains may be extracted from material contaminated by alien microflora by employing guinea pigs as live filters With this purpose, guinea pigs are inoculated intraperitoneally with 1 5 to 2 ml of kidney suspension After 10 to 30 minutes, blood is taken from the heart with sterile pipettes and inoculated into sterile cultural media, which in a few days will produce pure leptospire growths An advantage of the method is that the cultures include only the leptospire containing blood of the guinea pig without the initial kidney tissue of the animal under test, which may include antibodies hindering the growth of leptospires

To obtain pure leptospire cultures from carrier animals received dead (subjected to microbial seeding), resort may also be taken to laboratory bred wild rodents (young common hamsters and voles) putting them through microscopy and serologic tests for leptospire infection Ten to fifteen days later, the laboratory animals are inoculated subcutaneously with the materials (kidneys, urine) under test In positive cases, pure leptospire cultures may be isolated from the kidneys of the laboratory bred specimens

The cultures are kept in a thermostat for at least 30 days Every 5 days crushed drop samples are put to dark-field microscopy for presence of leptospires If such are revealed, even in small quantities, the material is immediately reinoculated into fresh media, subsequent reculturing being made not less than once in five days, since in many cases the primary specimens are extremely capricious, as it were, in culture on artificial media and require more care than museum strains until, so to speak, they become fully adapted to the medium

As mentioned earlier, when intended for serologic purposes blood drops from the animals under study are dried on filter paper This conservation method, which is likewise employed for other diseases, was first tested in diagnosing leptospirosis by ourselves in collaboration with I I Nikolayev in 1947-48 Agglutination lysis is staged not later than a month after desiccation The first dilution comprises 1/10 (2 drops of blood+18 drops of saline), or, in terms of serum, 1/20, then follow dilutions of 1/50, 1/100, 1/500, 1/1,000, 1/10,000, 1/50,000 and 1/100 000 For all sera under study, the agglutination lysis pilot test is staged only in the first two dilutions named (1/20 and 1/50) With positive results in these dilutions, the reaction is continued to serum titre For agglutination tests use is made of sev en- to ten day-old sera of museum strains including those known in the Soviet Union

Investigations for leptospire infection should be made shortly after the animal's death (24 to 72 hours, in summer), since the onset of microbial seeding handicaps isolation of pure cultures and subsequently leads to the latter's death and disappearance due to competition on the part of purogenic microbes Hence, for prompt treatment of material, the laboratory should be set up in the direct vicinity of the site of trapping Carcass investigation should be made, as a rule, within the first few hours, or at any rate, not later than 12 hours after the animals' death in snares

Identification of leptospire strains isolated from animals caught in nature is effected by the agglutination-lysis method Use should be made of museum strains (Table 1) The pathogenicity of the cultures for laboratory animals (guinea pigs weighing up to 100 200 grams) is determined as well

The bacteriological findings are utilised in pinpointing the location of the focus in question With this purpose, discoveries of infected and of healthy animals are plotted on a large-scale map by means of symbols, each of which represents a separate animal The best for use are large-scale land survey maps, which are available, as a rule, on all collective and state farms.

Mapping assists in rationalising exploration of infection reservoirs in nature Maps give a vivid idea of the distribution of an epizootic over an area, and in the case of operations covering several months, permit an estimate of seasonal changes in the size of the area affected by leptospirosis In this way it is possible to establish the season when the leptospirosis-afflicted area is of minimum size, i e, when the infected animals concentrate in so called elementary foci (N. P. Naumov) Such seasonal changes in focal area may vary broadly, as observed, for example, on the banks of lake Nero (Yaroslavl region) by V. V. Ananyin and E V. Karasyova in 1954, and may comprise from 20 hectares in spring to 800 hectares in summer, the elementary foci in the period of decline of the epizootic, i e, early in spring, presenting definite limited areas distinguished by increased density of *Microtus oeconomus* population In various landscapes and climatic zones, elementary foci may reveal certain distinctions as to time of formation and distribution over the area An exact estimate of the time of formation of elementary foci and the area they cover is essential for control campaigns The accomplishment of the latter precludes the spread of an epizootic to larger areas, thereby preventing outbreaks among people and agricultural animals

Control campaigns in natural foci may be effected by use of baits poisoned with zinc phosphide The lethal doses are established for every rodent species individually, the complete lethal dose comprising 2 mg for root voles (E V. Karasyova, E V Narskaya, D S Osherova, 1958), 12 mg for water rats (V V Kucheruk et al, 1955), 20 mg for susliks (N A Nikitina et al, 1955), etc

It is highly important that the chosen bait should be sufficiently attractive for animals of the given species A particularly good bait are wild plants, which are readily eaten by animals, e g, the roots of reeds and other water-plants, as in the case of water rats (V V Kucheruk et al) In common practice, the most extensive use is made of root crops and the seed of cultivated plants In particular, in foci of non icteric leptospirosis, whose main reservoir are root voles, control campaigns may be effected with the use of carrot bait (E V. Karasyova, E V. Narskaya, V V Ananyin, 1954) The recipe of the mentioned poison bait is as follows 100 gr carrot, 1 gr zinc phosphide, 2 gr vegetable oil Vegetable oil is used for applying the poison to the bait The carrots are cut into 1 gram slices, their amount being calculated in accordance with the total amount of poison One slice of carrot should hold 10 mg of zinc phosphide, or 5 lethal doses With lower concentration of the poison not all animals swallowing the bait will die since part of the bait may remain uneaten, or the poison be partly removed by the animals paws, etc On the other hand with larger concentrations, the bait proves less attractive for animals

The amount of bait to be used in campaigns per unit of area is determined by the density of the animal population in the given focus and the mean area of an animal s individual beat, i e, the size of the area which it inhabits The population density and individual habitation area may be estimated by marking (see above) The bait should be distributed in such a way that no less than 4 or 5 pieces occur on each individual plot With a mean density of 25 root voles per hectare 1 5 kg of poisoned bait will be required for a campaign In practice, the poisoning of an area is effected by workers moving in a row at 10 metre intervals from one another, laying 2 pieces of bait after every 2 or 3 metres Population counts should be made before and after the campaign to check its efficiency

The first attempt at using this method in a focus of leptospirosis near lake Nero (Yaroslavl region E V Karasyova et al, 1954), gave heartening results The root vole population in an elementary focus was completely destroyed, the rate of the epizootic, as compared with controls falling fivefold throughout the focal area and the effects continuing all through the epizootic season (from May to September incl)

In recent years, communications have been published on the incidence of leptospirosis among *Insectivora* Reservoirs have been revealed among common shrews, long eared hedgehogs (*L erinacei auriti*) European hedgehogs (*L erinacei europaei*) and water shrews (leptospires of the *L* poi *L sorex* type) The epidemiological importance of leptospirosis in *Insectivora* is not sufficiently clear Cases of infection with leptospires of the *L grippo typhosa* type have been revealed in polecats and certain oth ers. Also on record are high-lethal outbreaks caused by *L. pomona* among silver and arctic foxes on pelt farms. The epidemiologic importance of both wild and cultivated *carnivora* (foxes) is apparently not high.

As regards commensates, most important epidemiologically are leptospiroses of agricultural animals, chiefly cattle. The first to describe leptospirosis in cattle as a disease *sui generis* were S. N. Nikolsky, F. M. Desyatov and G. F. Marchenko (1935). In the Northern Caucasus these authors observed cattle diseases marked by jaundice and hematuria, which gave them reason to call it icterohaemoglobinuria. On the basis of detailed investigations on the course of the disease, as well as pathologo-anatomical, histological and epidemiological data, they concluded that, until its etiology is finally established, icterohaemoglobinuria should be regarded as a separate nosological entity. In 1939 the leptospirose etiology of the latter was established beyond doubt by V. I. Terskikh, M. V. Zemskov, S. Y. Kreutzer and Z. A. Roshchina, who succeeded in isolating pathogenic leptospire cultures from the blood of diseased animals. As demonstrated later, these cattle diseases are caused by the same species of leptospires as in man, i.e., by *L. grippotyphosa, L. pomona, L. tarassowi (L. mitis)* and, less frequently, by other species.

Our literature contains sufficiently detailed descriptions of the respective clinical picture, pathologic anatomy and histology. Suggestions have been put forward as to the sources and routes of dissemination of the infection, and measures have been proposed and carried out for its control. The clinical course of the disease in typical cases is sufficiently characteristic: short-term temperature rises (for 1-3 days) up to 40° or 41°C, mental depression, rejection of food, and frequently conjunctivitis. Simultaneously with the decline in temperature, the skin and visible mucous membranes become jaundiced, the urine acquiring a dark-red colouring. Most vividly manifest are jaundice of the sclera, udder, internal surface of the ear and other hair-less areas of the skin, necrosis is observed on sections of the skin on the back, udder, ears and elsewhere Lactation falls and the milk acquires a yellowish colour, containing admixtures of blood Lethality in icterohaemoglobinuria is quite high, especially in calves, comprising an average of 30 to 35 per cent In cases with a favourable outcome, convalescence is prolonged, often for many months due to developing cachexia, which frequently leads to inevitable slaughter According to I A Dukalov, the disease may also assume subacute, atypical and chronic forms V N Terskikh reports having observed leptospirose infection without any visible manifestations

Icterohaemoglobinuria, first recognised in the North Caucasus, proved to be a rather widespread infection in the south and east of our country In the central and northern regions of the Soviet Union it is met considerably rarer Cattle leptospirosis is also recorded in the U S A , and West-European countries like Switzerland, Germany, Bulgaria, Rumania and others, as well as Israel and Australia

It has been established that in animals recovering after icterohaemoglobinuria, the leptospires may survive in the kidneys and be eliminated with the urine The duration and intensity of leptospiruria are not sufficiently investigated V I Terskikh succeeded in isolating leptospires from the kidneys of calves slaughtered 3 months after icterohaemoglobinuria

Icterohaemoglobinuria is noted for its seasonal peaks The highest morbidity has been recorded in July-August September, i e , the months when people likewise most frequently contract the disease In contrast to the strict enough seasonal incidence of water fever and other leptospiroses in man a rather common occasion in epizootic foci are cases of icterohaemoglobinuria occurring in winter and spring (during stable maintenance) Infection may likewise occur throughout the year

Water is usually regarded as the main medium of transmission of icterohaemoglobinuria, the source being leptospire carrying agricultural animals which contaminate their watering and bathing places with urine This fact accounts for the observed higher incidence of the disease at pasturing as compared with stable maintenance (S N Nikolsky, F M Desyatov and G F Marchenko, I A Dukalov) Of greatest epidemiological importance in this regard is the watering of cattle in small stagnant ponds, swamps and shallow ' rotten rivers of the steppeland belt In settlements located along large river courses, i e, near running water, the disease is practically non existent At stable maintenance when animals are in close touch with each other transmission may be effected at contact between an animal s face or other part of the body and the urine of an eliminator of leptospires

Owing to the fact that leptospirosis in agricultural animals is observed to precede or coincide with outbreaks of water fever among people convalescent animals are usually regarded as a source of infection However, although admitting the considerable importance of cattle and other agricultural animals in the dissemination of leptospiroses among people, the part played by natural foci of the infection should not be underrated either

Epizootic outbreaks of cattle leptospirosis unassociated with natural foci are possible when animals from stricken farms are admitted into healthy herds in direct violation of sanitary and veterinary rules The duration of such epizootics in foci of anthropurgic character is directly dependent on the promptness of control It is well known that the first cases of icterohaemoglobinuria on hitherto satisfactory farms are registered after transferring the cattle from stables to pastures which in southern regions takes place in February March In foci of the disease cattle may be infected primarily, by leptospire carrying rodents which contaminate the reservoirs employed for watering and bathing cattle The existence of natural foci may serve to explain the maintenance of the infection in interepidemic and interepizootic periods

The incidence of the respective natural foci has been established in a number of regions where leptospirosis occurs among cattle, in particular, the Stavropol, Krasnodar and Primorye territories and Kazakhstan Leptospirosis in *pigs is mostly caused by leptospires of the species L pomona* The clinical symptoms here are generally obscure, including slight temperature rises and gastroenterites Leptospires are eliminated with the urine from the beginning of the third week which sometimes continues for up to a year Foreign authors, e g , Kathe (1950), assert that the *pomona* infection in swine owes its maintenance exclusively to infection of healthy animals by diseased, or, in other words, that swine are the principal reservoir and rodents only a side branch of the infection chain

The mentioned author, however, overlooks the high incidence of *L pomona* in grey rats and the latter's extensive contacts with swine (contamination of food and litter in pigsties, devouring of dead rats by swine, etc) Therefore, although it is impossible to deny the liability of swine infecting one another, rats should be considered as the main source of infection Outbreaks and sporadic cases were also described among people, who were associated with bathing in small reservoirs contaminated by infected swine Leptospirosis likewise occurs among other agricultural animals, e g , goats, sheep and horses, whose role in the epidemiology of the disease, however, is as yet not clear enough

Among domestic animals, especially important as a source of infection are dogs The disease in the latter is most often caused by two species *L canicola* and *L icterohaemorrhagiae* The infection may assume either a relatively mild or severe course, in the latter case with jaundice, hemorrhage and uremia Convalescent animals for a long time continue to eliminate leptospires with the urine, thus being a potential source of infection for man. The hazard of contagion from dogs is especially grave for personnel in dog-pounds and veterinary hospitals, as well as dog-tenders, owners of hunting dogs and pets. Leptospirosis most frequently occurs among strays, wherefore service dogs and pets should be prevented from associating with the former. In Indonesia leptospirosis has been recorded in cats. The causative agent in this case are two types: *L. batavia* and *L. javanica*. Cases of human contagion from pathogen-carrying cats are unknown.

Control measures against leptospirosis comprise the following:

(1) protection of people against contagion by all available means precluding direct or indirect contact with reservoirs as well as mass immunisation with preventive vaccines;

(2) measures in the natural and anthropurgic foci to eliminate the very possibility of infection by total sterilisation or destruction of the source.

The first category of control includes prevention of contamination of public water reservoirs (wells, water-mains, etc.) and food by rodents and other potential leptospire-carriers. To preclude contamination of open reservoirs by cattle, it is advisable to install special water-troughs and to appoint special places for watering and bathing cattle (e.g., on rivers—downstream from water-drawing places and human bathing sites). In foci of infection, the use of unsterilised water for drinking should be prohibited. Water decontamination, as is known, is effected by boiling or chlorination, the chlorine doses for the purpose not exceeding those used in the case of enteric fever and other intestinal diseases. Work in leptospirosis foci (haymaking, drainage, etc.), may be conducted only with occupational safety precautions, e.g., the use of waterproof boots and gloves. Extremely important in this respect is the prevention of abrasions and cuts of the skin, which may serve as portals of entry of the infection. Extensive mechanisation of haymaking and drainage are highly beneficial in preventing occupational diseases

Certain importance may likewise be ascribed to selective vaccination of sections of the population most exposed to leptospire infection (e g , herdsmen, milkmaids, veterinarians, slaughter-house workers, haymakers engaged on marshland, land reclamation and rat control personnel, bridge builders, water rat trappers, etc) A particularly effective measure is wholesale vaccination of cattle and other livestock in areas endemic for leptospirosis

Measures of the second category, which are aimed at elimination of infection sources, include rat control, eradication of leptospirosis among livestock, and swamp drainage Rat control, as a means of sanitation in natural foci of the disease, is not sufficiently used in practice The basic recommendations for sanitation of the natural foci in question through destruction of rodents by poisoned bait, were presented earlier in this chapter (p 208)

In anthropurgic foci, where the reservoirs of disease are agricultural animals, the principal sanitation measures are as follows (1) early diagnosis, (2) prompt isolation and treatment of diseased animals (eliminators being isolated until elimination ceases), (3) quarantines imposed on herds and flocks where cases of disease are recorded, (4) enforcement of necessary sanitary and veterinary precautions for stable and pasture maintenance

Drainage of stricken regions is the most radical means of sanitation, not only disrupting the principal chain of transmission (rodent—water—man), but providing the prerequisites for a drastic change in the environments of rodent reservoirs Drainage promotes the extermination of swamp vegetation, the main source of food for moisture-loving rodents (root voles, water rats), which must inevitably lead to the numerical reduction and subsequent complete extinction of the respective animals on the area

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TULAREMIA

Detailed studies on the distribution, maintenance and epidemiological manifestations of natural foci of tularemia are essential for the prevention of this disease, which logically envisages sanitation of the foci Academician Y N Pavlovsky's theory on the natural focality of diseases transmitted from animals to man, forms a sound theoretical basis for such studies Investigations on the natural foci of tularemia should be carried out with all due consideration for the economic influence of man (the social factor) In contrast to certain other diseases with natural foci, tularemia may exist despite the onset of agricultural development, irradiating into cultivated areas, and may even increase its effects when violations of agricultural standards are admitted

GENERAL INFORMATION

Tularemia is a disease caused by bacteria of the species *Francisella tularensis* McCoy et Chapin, transmitted to man by rodents, insects and ticks As classified by Y N Pavlovsky, tularemia belongs to the category of facultative transmissible diseases with natural foci The causative agent was first isolated in 1911 in the U S A by McCoy and Chapin (McCoy & Chapin, 1912) while investigating a plague-like disease in gophers The authors named the microbe *Bacterium tularense* after the district of Tulare, California on whose territory the diseased gophers were revealed Later, when the disease was discovered to afflict man, Francis (1921) proposed the term tularemia, based on the specific name of the causative agent In the U S S R, the microbe was first isolated in 1926 by S V Suvorov, A A Volfertz and M M Voronkova while investigating patients in the delta of the river Volga, near Astrakhan

The causative agent presents a well defined microbe form with marked parasitic features Some authors refer the tularemia microbe to the genus *Pasteurella*, others to *Brucella* The considerable distinctions of the pathogen from representatives of both genera give reason to agree with the proposal of K. A Dorofeyev (1947) that the microbe be placed into a special genus *Franciscella*

American strains of the microbe differ from European and Asian types in their greater pathogenicity for laboratory animals (especially rabbits) and man, as well as certain biochemical properties This indicates the existence of two geographic varieties of the microbe—American (New World or Nearctic) and Euroasiatic (Old World or Palaearctic) The Euroasiatic variety was recently nominated *Franciscella tularensis palaearctica*, whereas the American subspecies retains the original nomination of *F tularensis tularensis* (N G Olsufyev, O S Yemelyanova and T N Dunayeva, 1959)

Tularemia in man represents a febrile disease of infectional, allergic, and rarer, septic nature Usually, the disease continues from two to three weeks, sometimes more, and in rare cases may produce relapses occurring in the course of several years Direct contact with tularemia patients does not lead to infection Apart from general symptoms like fever, headache, debility, etc, tularemia is noted for inflammation of the lymphatic nodes and adjoining tissues (buboes), developing locally at the site of the pathogen's penetration into the body The lymphadenitis usually assumes a protracted form, and may be accompanied by suppuration of the inflamed lymphatic node (or nodes)

Clinical forms are distinguished by the site of the primary lesion, the following being known from practice ulcerous bubonic, bubonic, anginous bubonic, oculo bubonic, intestinal and pulmonary or thoracic (G P Rudnev, B N Stradomsky, A A Volfertz, A F Bilibin, I R Drobinsky, et al) As regards duration, tularemia may be acute, protracted and relapsing, and, according to degree of severity, mild, moderate and severe The most severe manifestations occur in the pulmonary form, while in the rest the course is moderate or mild Mean lethality in the U S S R and Western Europe is below 0 5 per cent in contrast to the U S A and Canada, where, without treatment, it comprises from 5 to 6 per cent, which is associated with the higher virulence of the pathogen occurring in America Autopsy reveals necrotic foci in the spleen, liver, lungs and other organs

Tularemia is marked by protracted recovery, with the possibility of remote relapses after various periods (up to a year and more)

The disease is known in America, Europe and Asia within the limits of the northern hemisphere, its incidence in Africa not being sufficiently proved Definite cases in the southern hemisphere are unknown

NATURAL FOCI

A natural focus of tularemia presents a biocenotic entity of complex structure, whose invariable components are the causative agent, vertebrate reservoirs and blood sucking arthropod vectors The infection may be disseminated among vertebrates not only by blood sucking arthropods, but by other routes as well, particularly through water and food

Investigation on natural foci of tularemia should cover the following (1) characteristics of focal terrain (2) teriological features (specific assortment, number and distribution of vertebrates) (3) parasitological characteristics (species, number and occurrence of blood sucking arthropods), (4) epizootological, and (5) epidemiological data

Focal Terrain

In contrast to the majority of diseases with natural foci, tularemia occurs on all types of terrain in every geographic zone on the non alpine areas of the U S S R, including tundra, forest, forest-steppe, steppe, and desert, occasionally even penetrating into the mountains However, as regards conditions for the maintenance of natural foci of the disease, the different zones are not equivalent Optimum environments exist in the forest steppe, steppe, and partly, hardwoods, as well as river floodlands and deltas, lake shores and swamps Within a zone, the foci are associated with definite habitats or biotopes

At the outset of investigations on a natural focus of tularemia a clear idea should be obtained of the topographic features of the harbouring area An essential requisite is a map of the locality, preferably with a scale of 1 200 000 or 1 500,000 With the latter's help, a survey of the area is made, taking special note of local geomorphology and hydrography, the character and distribution of vegetation, etc Depending on the varieties of habitats (meadows, fields, woods, etc) the area is divided into sections, these data being used for planning all further work in the focus Likewise, a brief geobotanical description of each habitat is to be furnished

At the nearest meteorological outpost, data are obtained, initially, on local climatic features, monthly meteorological reports being received in the course of subsequent work Terrain characteristics should likewise include data on local animal species These, however, being restricted to the limits required for analysis of the focus by the methods given below

In the U S S R , spontaneous tularemia infection has been revealed in 54 species of wild vertebrates including 32 rodents, 5 insectivores, 6 carnivores, 8 birds, 2 amphibians and 1 fish Besides that, tularemia has been recorded in 9 species of domestic animals (Table 1)

However, different species of animals show varying susceptibility and sensitivity to tularemia infection, their importance in the circulation of the pathogen in nature differing accordingly By the degree of their susceptibility and sensitivity to tularemia, modern authors divide vertebrates into three groups (N G Olsufyev and T N Dunayeva) The maintenance of natural foci of tularemia is restricted to a few widespread species of highly sensitive mammals (group I), e g , common voles, water rats, hares, domestic mice, hamsters, shrews, etc For these tularemia presents a severe septic infection, of which they usually die within 5 to 12 days after contagion The organs and tissues (including the blood) of diseased and dead animals contain an enormous quantity of tularemic bacteria, which are responsible for the primary importance of these animals as sources of infection Some species of rodents and insectivores, e g , susliks, marmots, grey and black rats, field mice, hedgehogs, etc , are low-sensitive to tularemia On receiving infection, they usually fall ill, yet their importance as sources of infection is limited (group II)

Finally, in such animals as cats, foxes, polecats and others, even on receipt of massive doses of the pathogen, the disease may proceed without visible clinical manifestations, whereas small doses are of no effect whatever (group III) The importance of such animals as sources of infection is null

For ungulates as well as birds and cold blooded vertebrates, the tularemia microbe is low pathogenic However, the importance of some of the aforesaid as sources of tularemia infection is not clear and requires further investigation

Table 1

Vertebrate Reservoirs of Tularemia in the U.S.S.R.[1]

Species	Year[2]	Authors	Place of discovery	Method
I. CLASS MAMMALIA				
Order *Rodentia*				
Fam. *Leporidae*				
White hare *Lepus timidus* L.	1931	I. I. Berezin	Kurgan	Epidemiologic observations
	(1935)	S. P. Karpov, N. I. Antonov (see S. P. Karpov and V. M. Popov, 1936)	Western Siberia	Isolation of culture
Brown hare *Lepus europaeus* Pall.	1935	S. N. Ruchkovsky et coll.	Ukraine	Epidemiologic observations
	(1940)	V. A. Ter-Vartanov et coll. 1943	Stavropol territory	Isolation of culture
Tolai hare *Lepus tolai* Pall.	(1941)	N. F. Kalachova et coll.	Jambul region, Kazakh S.S.R.	Ditto
Rabbit *Oryctolagus cuniculus* L.	1935	S. Y. Kreutzer, N. K. Grzhebina and I. S. Kvashnina	Rostov region	Ditto
Fam. *Sciuridae*				
Squirrel *Sciurus vulgaris* L.	1940	B. V. Voskresensky	Moscow region	Ditto
Small suslik *Citellus pygmaeus* Pall.	(1930)	P. K. Grishina (see D. A. Golov and V. I. Tiflov, 1934)	West Kazakhstan region	Ditto

Long tailed suslik *Citellus undulatus* Pall	(1941)	A A Seleznyova, 1949	Gorno Altaisk region	Ditto
Chipmunk *Eutamias sibiricus* Laxm	1938	S P Karpov	Western Siberia	Ditto
Fam Murinae				
Water rat *Arvicola terrestris* L	(1926)	S V Suvorov, V A Wolfertz, M M Voronkova, 1928	Astrakhan region	Epidemiologic observations
	1928	D A Golov et coll	West Kazakhstan region	Isolation of culture
Common vole *Microtus arvalis* Pall	(1936)	D A Golov (see N G Olsufyev 1941)	Near Alma Ata	Ditto
Ungur vole *Microtus maximowiczi* Schrenk	(1941)	T G Linnik, 1957	Transbaikalia	Ditto
Narrow skulled vole *Microtus gregalis* Pall	1941	S P Karpov et coll	Novosibirsk region	Ditto
Root vole *Microtus oeconomus* Pall	1941	S P Karpov et coll	Ditto	Ditto
Steppe lemming *Lagurus lagurus* Pall	1934	A L Kazantseva and V I Gorokhov	Volgograd region	Ditto
Musk rat *Ondatra zibethica* L	(1939)	A F Komarova, 1945	Novosibirsk region	Ditto
Bank vole *Clethrionomys glareolus* Schreb	1946	G P Slavin	Moscow region	Ditto
European pine vole *Pitymys majori* Thom	1949	M A Miroshnichenko and P D Golubev	Stavropol territory	Ditto

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[1] Disputable data not listed

[2] Figures without brackets denote year of publication figures in brackets—year of discovery, if publication was considerably belated

(Continued)

Species	Year	Authors	Place of discovery	Method
Northern mole vole *Ellobius talpinus* Pall	1957	P M Kucherov et al	Western Kazakhstan region	Isolation of culture
Domestic mouse *Mus musculus* L	1934	V A Berdnikov	Volgograd region	By passage through guinea pigs
Forest mouse *Apodemus silvaticus*	1946	G P Slavin	Moscow region	Isolation of culture
Yellow necked mouse *Apodemus flavicollis* Melch	1957	V P Borodin et al	Volgograd region	Ditto
Field mouse *Apodemus agrarius* Pall	(1940)	V N Ter Vartanov et al, 1942	Stavropol territory	Ditto
Harvest mouse *Micromys minutus* Pall	1940	B V Voskresensky	Moscow region	Ditto
Grey rat *Rattus norvegicus* Berk	1939	P V Somov	Rostov region	Ditto
Black rat *Rattus rattus* L	1955	Y A Myasnikov	Tula region	Ditto
Common hamster *Cricetus cricetus* L	1929	G I Zarkhi	Tyumen region	Ditto
Golden hamster *Mesocricetus raddei* Nehr	1954	M G Yakovlev et al	Rostov region	Ditto
Grey hamster *Cricetulus migratorius* Pall	(1940)	V N Ter Vartanov et al, 1943	Stavropol territory	Ditto
Tamarisk gerbil *Meriones tamariscinus* Pall	(1941)	P F Kalachova et al, 1957	Jambul region, Kazakh S S R	Ditto
Midday gerbil *Meriones meridianus* Pall	1957	M P Kucherov et al	West Kazakhstan region	Ditto

Fam. *Dipodidae*				
Great jerboa *Allactaga jaculus* Pall	1955	V P Romanova et al	Rostov region	Isolation of culture
Northern birch mouse *Sicista betulina* Pall	1955	V P Romanova et al	Rostov region	Ditto
Southern birch mouse *Sicista subtilis* Pall	1955	V P Romanova et al	Rostov region	Ditto
Order *Insectivora*				
Fam. *Talpidae*				
Mole *Talpa europaea* L	1940	B V Voskresensky	Moscow region	Ditto
Common shrew *Sorex araneus* L	1940	B V Voskresensky	Ditto	Ditto
Small shrew *Sorex minutus* L	1946	G P Slavin	Ditto	Ditto
Lesser white toothed shrew *Crocidura suaveolens* Pall	1950	Z M Protopopova and A V Feshchenko	Ditto	Ditto
Water shrew *Neomys fodiens* Schreb	1940	B V Voskresensky	Ditto	Ditto
Order *Carnivora*				
Fox *Vulpes vulpes* L	1956	A A Zaitsev	Stavropol territory	Ditto
Wolf *Canis lupus* L	(1940)	V N Ter Vartanov et al, 1943	Ditto	Ditto
Dog *Canis familiaris* L	(1940)	V N Ter Vartanov et al	Ditto	Ditto
Polecat *Putorius putorius* L	1940	B V Voskresensky	Moscow region	Ditto

(Continued)

Species	Year	Authors	Place of discovery	Method
Weasel *Mustela nivalis* L	1940	B. V. Voskresensky	Moscow region	Isolation of culture
Sable *Martes zibellina* L	1936	E. I. Kositsina and V. M. Popov	Western Siberia	Ditto
Mink *Mustela vison* Schreb	1956	M. F. Schmuter and S. G. Abramova	Ukraine	Ditto
Domestic cat *Felis catus* L	1940	G. Y. Sinai	Not noted	Ditto
Orders *Perissodactyla* and *Artiodactyla*				
Horse	(1938)	A. N. Uzunov (see B. V. Voskresensky, 1940)	Moscow region	Agglutination test
Cattle	(1938)	The same	Ditto	Ditto
Sheep	1938	K. A. Dorofeyev and V. I. Gorokhov	Volgograd region	Ditto
Swine	(1938)	A. N. Uzunov (see B. V. Voskresensky, 1940)	Moscow region	Ditto
Camel	1934	D. A. Golov et al	West Kazakhstan region	Isolation of culture
II CLASS *AVES*				
Hen	(1940)	V. N. Ter-Vartanov et al., 1943	Stavropol territory	Isolation of culture
Kite *Milvus korschun* Gmel	(1940)	The same	Ditto	Ditto

Pigeon *Columba* sp	(1940)	The same	Ditto	Isolation of culture
Capercaillie *Tetrao urogallus* L	1957	I G Rubanova	Byelorussia	Ditto
Moor hen *Gallinula chloropus* L	1955	I G Rubanova	Byelorussia	Ditto
Corncrake *Crex crex* L	1956	I I Kleitman and N I Igolkin	Western Siberia	Ditto
Tern *Sterna hirundo* L	1956	The same	Ditto	Ditto
Quail *Coturnix coturnix* L	1956	The same	Ditto	Ditto
Black headed gull *Larus ridibundus* L	1946	G P Slavin	Moscow region	Ditto

III CLASS *AMPHIBIA*

Lake frog *Rana ridibunda*	1949	M A Miroshnichenko and P D Golubev	Stavropol region	Ditto
Toad *Bufo viridis*	1949	The same	Ditto	Ditto

IV CLASS *PISCES*

Loach *Nemachilus dorsalis*	1950	V P. Bozhenko	South Eastern Kazakhstan	Ditto

In natural foci of tularemia, sheep, cattle, swine and other domestic animals may contract the disease, which however, assumes a mild course (mostly latent), whence they cannot maintain the circulation of the pathogen in natural foci Indirectly, however, they encourage it by serving as hosts for mature ticks, vectors of tularemia

In the light of modern knowledge on the importance of different vertebrate species in the circulation of the tularemia pathogen in foci, investigations in the latter should primarily be concentrated on the study of rodents and insectivores The studies should reveal the specific assortment of the local fauna, listing each species and its percentage in the total animal population, and should establish the qualitative and quantitative distinctions in the population dynamics of the most widespread forms (amplitude and timing of variations, etc) Specific assortment and population data are collected separately for all the different habitats comprising the area under survey

Special emphasis in the studies should be laid on mass spread species of rodents and insectivores highly sensitive to tularemia (group I) The animals are captured by snares spring traps, cage traps, etc , set out in rows of 25 and more in the respective habitats (Fig 26) The population is estimated by the percentage of captures per night Especially effective means of capturing small animals are ditches (Fig 27) furnished with metal cylinders from which the animals cannot escape

Investigations on the population and distribution of animals in their principal habitats are conducted two or three times during the snowless season, providing the data for estimating numerical fluctuations in the animal population In regard to certain species of rodents, e g , common voles, these data may be obtained by counting burrows, determining (by excavation) the percentage of burrows inhabited by rodents In winter, the population of Muridae may be assayed by overhauling hayricks (Fig 28) and

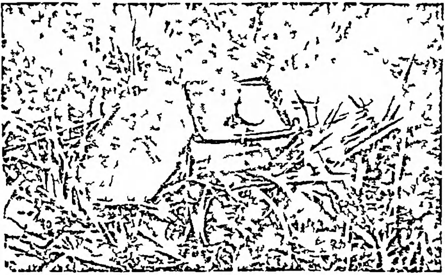

Fig 26 Water rat caught in spring trap (N G Olsufyev)

Fig 27 Trap pits for rodents and insectivores (N G Olsufyev)

Fig 28 Winter overhaul of strawstack for collection of rodents (N G Olsufyev)

Fig 29 Rodent autopsy in laboratory

chaffheaps in the fields, calculating the number of animals caught per cu m of substratum

The obtained animals are subjected to autopsy and bacteriological study (Fig 29), noting the age and germinant state of the specimens, the number of embryos in females, etc These studies are augmented by various other eco logic observations

*Carefully collected for a number of years, such informa*tion on the specific assortment, distribution and population dynamics of small mammals is highly important for draw ing epizootologic conclusions The data are plotted on maps, graphs, etc On the basis of collected materials, the area is divided into districts, noting the localities identical as to the dynamics of background species, drawing up draft forecasts on the expected population rates of epidemiologically hazardous rodent species, etc

Parasitology

In rodents and other mammals, tularemia is mostly transmitted by blood sucking arthropods, in particular, ticks, lice, mosquitoes, and, to a smaller degree, fleas Of greatest importance as pathogen vectors are *Ixodidae*, and, to some extent, *Gamasidae*, in whose bodies the pathogen can reproduce and survive for long periods Due to that, ticks act as the principal reservoirs of tularemia in the natural foci of this disease In the U S S R, spontaneous tularemic infection has been revealed in 45 species of blood sucking arthropods, including 14 *Ixodidae*, and 6 *Gamasidae*, as well as 6 species of fleas, 10 mosquitoes, 5 *Tabanidae*, etc (Table 2) Besides that, the tularemia pathogen has been discovered in 13 species of non sangui vorous invertebrates (*Trichoptera, Crustacea, Mollusca,* etc), *or so called hydrobionts, in which spontaneous in*fection is usually associated with water contaminated by tularemia afflicted rodents

Table 2

Invertebrates Revealing Spontaneous Infection with the Tularemia Pathogen[1]

Species	Year of publication	Authors	Place of discovery	Note
		I ARTHROPODA		
		A *Arachnida*		
1 Ixodidae				
Ixodes apronophorus P Sch	1934	D A Golov	Near Alma Ata	Larvae and females taken from diseased water rats
Ixodes ricinus L	1943	N G *Olsufjev*	*Moscow region*	Mature ticks from cattle
Ixodes persulcatus P Sch	1944	S P Karpov and V M Popov	Western Siberia	Mature ticks from domestic animals
Ixodes laguri Olen	1949	V P Romanova and K I Krivonosov (see Krivonosov 1949)	Rostov region	
Dermacentor marginatus Sulz	1934	D A Golov[2]	Near Alma Ata	Nymphs from water rats and common voles
Dermacentor pictus Herm	1940	N G Olsufjev and M V Afanasjev (see Olsufjev 1940)	Moscow region	Mature ticks from pastures and agricultural animals
Dermacentor silvarum Olen	1944	S P Karpov and V M Popov	Western Siberia	Mature ticks from cattle
Dermacentor nuttalli Olen	1947	I Y Olkhovik A A Leontjev and I T Shvedko	Transbaikalia	Mature ticks from domestic animals

Haemaphysalis punctata Can et Fanz	1953	Y F Shatas and N Y Bistrova	Volgograd region	Mature ticks from pastures and cattle
Haemaphysalis otophila P Sch	1955	V G Petrov and L A Pshenichnaya (see V V Kucheruk V G Petrov et al 1955)	Stavropol region	Mature ticks from pastures
Haemaphysalis concinna C L Koch	(1957) 1959	N G Olsufyev V G Petrov and V V Kucheruk 1959	Altai territory	Mature ticks from pastures
Rhipicephalus rossicus Yak et K Yak	1953	Y V Shatas and N Y Bistrova	Volgograd region	Mature ticks from cattle hedgehogs hamsters and water rats
Rhipicephalus pumilio Pom	1957	N F Kalachova P I Kamnev and A D Lukyanova	Jambul region	Ticks from hares tamarisk gerbil dog and calf
Hyalomma plumbeum Panz	1955	V G Pilipenko and K I Derevyanchenko	Astrakhan region	Nymphs from hares
2 Gamasidae				
Haemolaelaps glasgowi Ewing	1951	E N Nelzina and I P Barkov	Rostov region	
Laelaps muris Ljung	1951	The same	The same	

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[1] Disputable statements not listed

[2] D V Golov erroneously calls it *D silvarum*

(Continued)

Species	Year of publication	Authors	Place of discovery	Note
Mixture *Hypoaspis sp Haemolaelaps nidi Laelaps stabularis*	1940	N G Olsufyev	Moscow region	From nest with fresh tularemia killed carcass of common vole
Macrolaelaps multispinosus Banks	1957	T G Linnik	Transbaikalia	Ticks from musk rats
B. Insecta				
1 Bugs				
Cimex lectularius L.	1940	G Y Sinai	Not indicated	
2 Lice				
Hoplopleura sp	1952	I I Novikova	Astrakhan region	From water rat
3 Fleas				
Ctenophthalmus assimilis Tasch	1940	N G Olsufyev	Moscow region	From nest with remains of common voles on area of tularemia epizootic
Ctenophthalmus acuminatus Joll et Arg	1949	V P Romanova K I Krivonosov (see Krivonosov, 1949)	Rostov region	From golden hamster

Ctenophthalmus pollex Wagn et Ioff	1934	A A Wolferts S A Kolpakova and A V Flegontova	Volgograd region	From empty nests of common voles and steppe lemmings
Ceratophyllus consimilis Wagn	1953	V N Ter Vartanov et al	Stavropol territory	From vole nest in area of intense tularemia epizootic
Ceratophyllus calcarifer Wagn	1957	O F Pauler Y G Shvetsov and I P Potapova	Transbaikalia	
Ceratophyllus penicilliger Gr	1956	V M Popov and O K Kupressova (see V M Popov and V I Igolkin 1956)	Western Siberia	From water rats
4 Diptera				
(a) Mosquitoes				
Aedes vexans Mg	1951	E I Novikova	Astrakhan region	
Aedes cinereus Mg	1953	S P Karpov V M Popov A G Slinkina et al	Western Siberia	
Aedes excrucians Walk	1953	The same	The same	

(Continued)

Species	Year of publication	Author	Place of discovery	Note
Aedes communis Deg	1950	V. M. Popov and O. K. Kupressova (see V. M. Popov, 1950)	Western Siberia	
Aedes punctor Kirby	1950	S. P. Karpov and V. M. Popov	The same	
Culex modestus Fic	1948	I. I. Novikova	Astrakhan region	
Culex pipiens L.	1955	V. P. Romanova, V. P. Bozhenko and M. G. Yakovlev	Rostov region	
Anopheles hyrcanus Pall	1951	I. I. Novikova	Astrakhan region	
Anopheles maculipennis Mg	1951	I. I. Novikova	The same	
Anopheles bifurcatus L.	1955	F. G. Rubanova	Byelorussia	
(b) Culicoidae				
Culicoides pulicaris L.	1943	S. P. Karpov, V. M. Popov, A. G. Slinkina et al (for precise nomination see V. M. Popov, 1949)	Western Siberia	

(c) Midges				
Eusimulium pusillum etc [1]	1951	T A Rubina	Perm region	
(d) Tabanidae				
Chrysops relictus Mg	1940	P V Somov and V P Romanova	Rostov region	
Tabanus autumnalis L	1946	E I Novikova	Astrakhan region	
Tabanus flavoguttatus Sz	1953	V G Pilipenko	The same	
Tabanus bromius L	1951	T A Rubina	Perm region	
Chrysozona pluvialis L	1940	P V Somov and V P Romanova	Rostov region	
5 Trichoptera				
Limnophylus stigma Curt	1953	A A Seleznyova	Western Siberia	Larvae from infected water
Limnophylus rhombicus L	1956	The same (see S P Karpov and V M Popov 1956)	Western Siberia	The same
Anabolia sororcula McL	1956	The same	Western Siberia	The same

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[1] T A Rubina mentions midges but Y G Mitrofanova who took part in these expeditions, states in her reply to our request that the proportion of midge species in the collections was as follows *Eusimulium pusillum*—77 4 per cent *Simulium morsitans longipalpe*—12 per cent *S ornatus*—4 per cent, *Eusimulium erythrocephalus*—2 7 per cent other species—3 9 per cent The lots were studied for infection without division into species

(Continued)

Species	Year of publication	Authors	Place of discovery	Note
		C. CRUSTACEA		
Gammarus pulex	1950	A. A. Seleznyova	Western Siberia	From infected waters of a stream
Gammarus balcanicus Schäf.	1957	N. G. Olsufyev, V. V. Kucheruk and V. G. Petrov	Altai territory	The same
Potamon potamios Oliv.	1950	O. V. Ovsapyan	Armenia	
		II. MOLLUSCA		
Radix pereger Muller (*Limnaea peregra*)	1950	V. P. Bozhenko	Eastern Kazakhstan	From stream water
Radix (*Limnaea*) *ovata* Drap.	1953	A. A. Seleznyova	Western Siberia	The same
Galba (*Limnaea*) *truncatula* Mull.	1950	A. A. Seleznyova	The same	The same
Pisidium casertanum Pol.	1956	A. A. Seleznyova (see S. P. Karpov and V. M. Popov 1956)	The same	The same
Anisus (*Planorbis*) *contortus* L.	1950	A. A. Seleznyova	The same	The same
Gyraulus (*Planorbis*) *albus* Mull.	1953	A. A. Seleznyova	The same	The same
		III. ANNELIDA		
Leeches	1935	S. N. Ruchkovsky et al.	Ukrainian S.S.R.	Epidemiological data

Surveys of tularemic foci should include evaluation of the specific nomenclature and population of the most wide spread groups of blood sucking arthropods, including the ectoparasites of wild rodents and *Insectivora* (ticks, fleas, lice) as well as freely existing forms attacking rodents and man (mosquitoes, horse flies, certain species of ticks and mites, etc) Ticks, fleas and lice are collected when inspecting animals and their nests To prevent the escape of ectoparasites, immediately on capture, the animals are placed into cotton bags, firmly tying them with string

Blook sucking *Diptera* and *Ixodidae* are collected in the habitats of predominant concentration of mass spread species of rodents highly sensitive to tularemia (group *I*), which is effected either by catching them free, or by collection from domestic animals Priority is given to the study of *Ixodidae* whose larval and nymphal stages parasitise rodents and insectivores and blood sucking *Diptera* (horse flies and mosquitoes) as being the most important vectors of tularemia

Ectoparasite population counts are conducted on animals and in burrows with all possible regularity, immediately on the receipt of material from the zoologist Population counts concerned with mature *Ixodidae* parasitising domestic animals are conducted once weekly, from the appearance of the first ticks until their total disappearance Commonly, the ticks appear on cattle in spring, in the first week after the beginning of pasturing Collection on pastures is conducted by means of flags and accomplished as a one-stage operation, with the utmost expediency The season for collection is spring, the tick period of activity in nature Counts of blood sucking *Diptera* are made once in a decade throughout the warm season Mosquitoes are caught with conventional entomological nets, which are waved around the observer, the number of insects caught per one hundred strokes being taken as a unit

Horse flies should be captured beside a horse or bullock the counting unit being the number of insects caught per 20 minutes

The collected blood sucking arthropods are subjected to bacteriological study, leaving part of the specimens for sample displays, for which ticks, fleas and lice are placed in 70° alcohol, mosquitoes and sandflies are stuck on pins, etc

The annual fluctuations in the parasite population are confronted with those of their hosts, weather changes, etc

Epizootology

In tularemia, as in the plague and certain other diseases with natural foci, the motive power of the epizootic process are the population level and mobility of the rodents sensitive to the given disease and the arthropod vectors responsible for maintaining contact between the animals at the rate necessary for the development of the epizootic This process takes place in concrete environments by which it is directly or indirectly influenced (Table 2)

Widespread epizootics of tularemia are observed among rodents only when the latter s population is sufficiently high the higher the population, the greater the scope of the epizootic In years when the rodent population is low, the disease occurs only sporadically, or for some time may even completely disappear from the rodent population, surviving in ticks

The spread of tularemia over an area is marked by fluctuations connected with the seasonal and perennial changes of the rodent population In various types of foci, the seasonal incidence and scope of epizootics may vary, depending, firstly, on the ecologic peculiarities of the local mass spread rodent species highly sensitive to tularemia (group I), and, secondly, upon the routes of transmission

Investigations on the epizootologic features of a natural focus should envisage the following (1) organisation of expeditions to locate the epizootic, (2) bacteriological study of small mammals, blood sucking arthropods and samples of environmental objects obtained in the focal area under survey, (3) determination of the virulence and other properties of tularemia strains isolated in the focus, (4) experimental assays of the susceptibility and infectional sensitivity to tularemia of the wild vertebrates most common in the focus, (5) experimental evaluation of the ability of local blood sucking *Diptera* suspected as vectors to transmit and maintain the pathogen, (6) experimental research on other routes of transmission

Detection of epizootics Sample collections intended for revealing epizootics of tularemia are made in a focus during planned teriologic and parasitologic surveys (see above) or specially organised campaigns The procedures employed are as follows

(a) Capture of animals and collection of carcasses in localities with the highest animal populations at the dates most favourable for the development of epizootics, e g, for water rats—in spring and summer during floods, for mice and voles—in autumn, winter, and early in spring when they concentrate in hayricks and stacks, for rodents of many other species (voles, water rats, harvesters, etc) —in July August at the time of mass infestment with ixodid ticks, etc

(b) Collection of adult *Ixodidae from* pastures or cattle in areas with high populations of the rodents most sensitive to tularemia (group I)

(c) Collection of mosquitoes, horse flies and other blood sucking *Diptera* on the sites of suspected epizootics

(d) Regular obtainment of water-samples from sources arousing suspicions as to the presence of the tularemia pathogen

(e) Collection of rodent nests, sample surveys of straw, chaff, etc, from stacks and ricks in winter time liable to harbour epizootics of tularemia among rodents

An important indication of the presence of a tularemia epizootic among rodents are reports on the discovery of mouse carcasses while threshing or transporting hay, testimonies of a murrain among water rats, musk rats, hares and other game rodents, received from the population, hunters, pelt depot personnel, and reported cases of tularemia among people

When conducting special purpose surveys for the discovery of infection, the methods of capturing wild animals for bacteriological study should be as varied as possible to include, besides trapping and shooting (e g , of hares), *excavation of burrows*, ditch snaring, flooding, and over haul of stacks and ricks Captured animals belonging to the group most sensitive to tularemia, such as water rats or voles, are preferably delivered alive for 15 day laboratory observations, which increases the yield of positive results Likewise essential are special searches for wild animal carcasses involving thorough examination of the terrain or overhauled ricks and stacks

Systematic epizootological surveys of the focal area offer an idea of the regularities governing the circulation of the pathogen therein, which is important for epizootological prognosis, as well as preventive measures Prompt detection of tularemia epizootics among rodents is vital for adopting urgent anti epidemic measures among the population

Bacteriological study The tularemia pathogen is referred to microorganisms of the first group, due to which its isolation and identification may be effected only on specially equipped laboratory premises affiliated to the departments of high hazard diseases at regional (territorial) sanitary and epidemiological stations, anti plague stations and research institutes, or in adequately fitted field laboratories by specially qualified personnel Delivery of the material (rodents, blood sucking arthropods etc) for laboratory bacteriological study is effected with all precautions stipulated for work with high hazard diseases Serologic studies for tularemia may be conducted in any diagnostic laboratory

Investigations for tularemia infection in vertebrates blood-sucking arthropods or environmental objects are conducted mainly with the use of bacteriological methods such as inoculation into live animals, culturing and bacterioscopy Immunological techniques include precipitation tests, agglutination or allergy tests being comparatively seldom used for investigations on wild animals (especially rodents)

When conducting tests for tularemia, the possibility of revealing other rodent diseases like erysipeloid, listerellosis, pseudotuberculosis, salmonellosis, etc, should also be accounted for Isolation of the respective pathogens may be effected during bacteriological studies for tularemia

Bacterioscopy Owing to the minute dimensions of the tularemia microbe (0 3 to 0 5 μ), it may be detected with certainty only at abundant microbial seeding of the material under study Bacterioscopy may be used in studying the organs and tissues of dead wild or laboratory animals, but in freshly-killed specimens it generally proves useless It is likewise inapplicable for water, straw washings etc

Smears are stained by the Romanowsky Giemsa method, which is best for the purpose, or by the Gram technique On stained organic preparations, the tularemia microbe appears as extremely minute cocci or cocco bacteria The organism evinces a marked tendency to clumping In animals of the first group (voles, water rats, domestic mice, etc) dying after tularemia, bacterioscopy of smears from the spleen, liver, blood, etc, reveal enormous concentrations of the microbe The tularemia pathogen is Gram-negative, i e, stains pink, the Romanowsky Giemsa stain producing a deep violet In tissue staining, tularemia microbes do not stain bipolarly, which distinguishes them from *Pasteurella*

The amount of tularemic bacteria revealed in organic smear prints and blood smears, is recorded by the following scale (D A Golov and N G Olsufyev) continuous colonies or numerous large groups of bacteria at each view—IV points, numerous medium- and small sized groups at

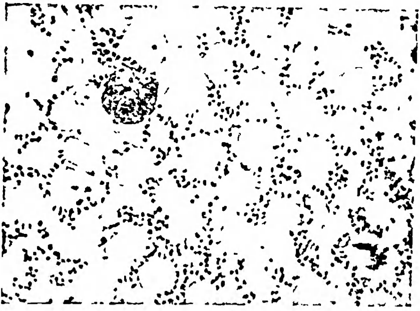

Fig 30 Tularemic bacteria in blood of water rat killed by disease in nature Amount of bacteria—up to III points (by scale described in the text) (V. V. Kucheruk)

each view—III points; solitary bacteria and small groups at each view—II points; solitary specimens and small groups at some views—I point.

By use of bacterioscopy (combined with precipitation) positive results may be obtained in as soon as two or three hours pending receipt. The obtained data, however, are merely preliminary, since positive bacterioscopic findings and precipitation tests must be checked by isolating the respective cultures.

Culturing. This method is chiefly used for isolating tularemia cultures from the organs of received dead wild animals, as well as laboratory specimens either dead or killed after live-test experiments. The material used are portions of the spleen, liver, hypertrophic lymphatic nodes, or blood. For *Ixodidae* this method is less fruitful owing to frequent contamination of the cultures with alien bacteria.

It is inadvisable to employ culturing for wild animals caught alive and revealing no marked pathological changes at autopsy The organs of such animals are subject only to biological study Water, soil, food washings, etc, are not tested by culturing due to contamination with alien bacteria, inhibiting the growth of the tularemia pathogen which is a rather exacting microbe when cultured on artificial media Thus, for example, it refuses to grow on conventional meat peptone agar or broth The microbe may be cultivated on yolk media, while in agars it will reproduce only with addition of cystine and other nutrients, especially blood Such fastidiousness towards culture media is perhaps explained by limited choice or specialisation of ferments, due to which a number of substances, particularly amino acids (cystine) are utilised by the microbe only in "prepared" form

The optimum growth temperature is 36° to 37°C The microbe is a strict aerobe Cultures are mostly obtained on curdled yolk media prepared after McCoy out of chicken egg yolks (60 per cent) and saline (40 per cent), which are poured into test tubes and curdled during an hour at 80° C At profuse inoculation, tularemic bacterial growths in such media appear as thin continuous, shining efflorescences already after 18 to 24 hours of incubation in a thermostat at 37° C, and in 2 or 3 days reach maximum

When inoculated sparely, isolated colonies become noticeable after 3 to 5 days and more Curdled yolk media are used not only for culturing tularemia microbes from animal organs but also in maintaining microbe cultures for museum and routine laboratory purposes, the microbes in such media being less apt to dissociate Reculturing is carried out every one and a half months, storing the cultures on ice M S Drozhevkina (1945) proposed a liquid yolk medium (10 per cent yolk and 90 per cent sterile saline) for culturing tularemia microbes As regards sensitivity, the aforesaid reveals no advantage over a properly prepared McCoy medium, due to the former s intransparency, the presence of bacteria may only be detected by reinoculation into the latter (T N Dunayeva and O S Emelyanova)

Nor have blood media any advantage over curdled, yolk When culturing pieces of visceral tissue, especially by the so called print method, solitary bacteria will survive in yolk media as well

When receiving fresh rodent carcasses (e g , in winter), *Franciselfa* cultures may be obtained in yolk media on the very next day

Identification is made on the basis of a combination of the following indices (1) morphology and staining of bacteria in smears (2) features of growth on curdled yolk media, (3) absence of growth on peptone agar and broth (4) agglutination by specific serum up to own titre or up to half of the latter s, (5) pathogenicity for laboratory animals (ability of the culture on test to cause death of white mice or guinea pigs on injection with organic changes typical of tularemia and subsequent isolation of pure cultures)

Isolation of the culture (following identification) furnishes data for the final conclusion

In vivo tests are the most sensitive and reliable method of detecting tularemia in any material to be tested The test may be applied to the organs of dead or killed wild mammals, blood sucking arthropods water, washings from various substrata, etc The subjects are white mice and guinea pigs into which the material (suspended in saline) is introduced subcutaneously or intraperitoneally The infection is lethal even when the suspension contains only solitary *Francisella* Bacterioscopy of blood and organic smears from the dead mice reveals enormous quantities of the pathogen (up to III or IV points), whereas guinea pigs may prove negative Tularemic cultures from the spleen, lymphatic nodes, etc , of animals dying after the disease, especially white mice, are readily cultured on media If the latter fails, the test is repeated on a white mouse, injecting a suspension from the spleen of the dead animal Isolated cultures are identified by the indices already mentioned

Precipitation Generally used for animals dying of tularemia The procedure is similar to Ascoli's, a high (1 6,000 to 1 8,000) titre agglutinative tularemia serum being used The crushed liver or spleen are suspended in saline, boiled for 15 to 20 minutes on a waterbath, filtered through asbestos wool, and, after adding serum, subjected to precipitation Positive results are usually obtained with organs microscopically revealing large amounts of bacteria Hence, negative precipitation results do not prove the total absence of *Francisella* in the material tested A positive reaction must be confirmed by isolating tularemia pathogens from inoculated cultures and bacterioscopy In some cases the reaction may be non specific

Agglutination In various species of animals, as also in man, the disease leads to the development in the blood of antibodies specific for *F tularensis* These antibodies may be revealed by agglutination

Generally, the serum agglutination titres for wild animals recovering from tularemia are not high, e g , 1 10 to 1 20, seldom 1 80 and more in grey rats, 1 20 to 1 80 and more (up to 1 400) in susliks, 1 10 to 1 40 in hedgehogs, and even 1 5, as in the field mouse After two to three months the amount of agglutinines falls so drastically that they may not be detected

To obtain the serum required for the test, wild animals are delivered to the laboratory alive, taking their blood from the heart under ether anaesthesia or during autopsy The test is staged only on large specimens of species in which tularemia is commonly benign (groups II and III), *e g , susliks, domestic rats, hedgehogs, cats, foxes, etc* Agglutination is done by volume, the initial dilution being 1 5 The antigen used is the *tularemia diagnosticum* or a live virulent culture of the bacteria (preferably the latter) Prior to mixing the antigen with the diluted serum, the former is diluted to a concentration of 1,000 million microbe cells per ml, the subsequent procedure being as usual The results are considered positive if in one or several of the serum dilutions agglutination is followed by total or almost total clarification and the appearance of a dense sediment (agglutinane) which on shaking decomposes into large flakes (Vi agglutination) If none of the dilutions produce clear agglutination (four or three plus) the result should be regarded as doubtful

Allergy tests Wild animals delivered to the laboratory alive may be tested for tularemia by their allergic reaction This diagnostic method is practicable chiefly for relatively large animals, e g , susliks, marmots, etc , but inadvisable for *Muridae*

Tests are effected with tularin containing 1,000 million microbe cells per ml, i e , ten times more than the usual preparation for subcutaneous injections The fur on the animal's back or thigh is sheared off, and with a thin needle 0 1 ml of the antigen is introduced strictly subcutaneously The results are evaluated first after 24 hours, and finally, after 48 In positive cases, a palpable infiltrate 0 3 to 0 5 cm and more in dia appears at the site of the injection The appearance of a smaller infiltrate after 24 hours and its complete disappearance towards 48 is regarded as a doubtful reaction

It should be borne in mind that several days after the tularin injection, antibodies against the tularemia pathogen appear in the animal's serum Therefore, to reveal antibodies testifying to natural infection, it is necessary to take the animal's blood prior to the allergy test

Properties of isolated strains The problem of the tularemic pathogen's variability in natural environments constitutes an important aspect of the epizootology and epidemiology of this disease Of greatest interest in this respect is the pathogen's virulence, although the potential variability of other properties should be considered as well To this day, no distinctly different serotypes of *F tula* *rensis* have been reported, nor have any serologic distinctions been found between its American and Eurasian varieties

The strains of tularemic bacteria isolated from rodents, ticks, etc, as well as human patients in natural foci of the U S S R, are closely similar in all features, including virulence Literary statements have been made on the isolation of so called atypical strains of tularemic bacteria, capable of growing on simple culture media, and morphologically roughly representing bacilli, etc (P V Somov and N N Lange, 1950, et al), which however on later test were found to be alien bacteria, mostly saprophytes

Geographic surveys of tularemic strains occurring in different points and objects in the U S S R have been insufficient and should be carried further

The strains obtained should be studied with regard to their morphological, tinctorial, cultural, biochemical, antigenic and virulent properties Since the virulence of bacterial strains is of primary importance in studying epizootological and epidemiological regularities, special attention should be devoted to its study Virulence should be tested not later than a month after isolation, since in maintenance (with reinoculation) on artificial media, the cultures show a tendency towards gradual attenuation

The strains are tested on laboratory animals Subcutaneous injections are employed to estimate the minimum complete lethal dose (DCLM), or incomplete lethal doses (e g, LD_{50}) The suspension to be used is prepared from a two day culture from curdled yolk media The initial suspension is made for a concentration of 1,000 million per ml, comparing it for transparency with the optical standard of the U S S R State Control Institute, and diluting it further by 10, 100, etc, times, each time using a fresh pipette

The fullest estimate of a strain's virulence is obtained in simultaneous tests on animals revealing various resistance to tularemia white mice and guinea pigs (highly sensitive animals of group I) white rats and rabbits (group

II—low sensitive) The former should be infected with doses of 0 1-1-10 microbe cells the latter—with 10 100 million to 1,000 million Five white mice or three other animals are taken per dose using adult specimens of normal weight With difficulties in obtaining animals, the experiments may be staged on white mice, white rats and guinea pigs, or, in extreme cases, on the first two alone Diagnosis of tularemia in dead experimental animals should be confirmed by pathologo anatomic study and isolation of the initial culture In certain cases it may be confirmed by positive bacterioscopy of smears

The susceptibility and infectional sensitivity of vertebrates to tularemia has at present been assayed experimentally for 70 representatives of the vertebrate fauna of the U S S R , including 54 rodents, 9 insectivores, 6 carnivores, and one species of cold blooded vertebrates Most of the investigated rodents (38 species) and certain insectivores (4 species) proved highly susceptible and highly sensitive to tularemia (group I), while the other rodents (16 species) and insectivores (5 species) are highly susceptible but low sensitive (group II), all the investigated wild carnivores (6 species) and the one species of frog put to study being low susceptible and practically insensitive (group III) (Table 3)

Of the domestic animals, only two may be referred to definite groups the rabbit (group II) and the cat (group III) Sheep, apparently, belong to group II, cattle horses etc to group III This problem, however, requires additional research The same characteristics for wild birds have not been sufficiently studied, yet the fragmentary experimental data available permit to conclude that they belong to groups II and III

The susceptibility and sensitivity of individual vertebrate species to the more virulent American strains, may not be the same as to Eurasian strains Thus as regards sensitivity towards American strains the rabbit belongs to group I

Table 3

A Mammal Species Experimentally Referred to Group I
(highly susceptible and highly sensitive to tularemia)

Species	Authors and date
Rodents	
White hare—*Lepus timidus* L	N G Olsufyev T N Dunayeva et al, 1950
Brown hare—*Lepus europaeus* Pall	T N Dunayeva, 1954
Musk rat—*Ondatra zibethina* L	T N Dunayeva and O S Emelyanova, 1950
Water rat—*Arvicola terrestris* L	G I Zarkhi, 1930 D A Golov, 1934
Common vole—*Microtus arvalis* Pall	D A Golov, 1934
Field vole—*Microtus agrestis* L	N. G Olsufyev, T N Dunayeva et al, 1950
Root vole—*Microtus oeconomus* Pall	T N Dunayeva, 1954
Reed vole (Far Eastern)—*Microtus fortis* Büchn	N D Altaryova and E A Milina, 1945
Narrow skulled vole—*Microtus gregalis* Pall	E P Kleitman, 1955
Short tailed vole—*Microtus socialis* Pall	T N Dunayeva and N G Olsufyev, 1952
Brandt s vole—*Microtus brandti* Radde	L A Smirnova and L V Vasyukhina, 1951
Streltzov s vole—*Alticola strelzovi* Kastsch	T N Dunayeva
Bank vole—*Clethrionomys glareolus* Schreb	N G Olsufyev, T N Dunayeva et al, 1950
Large toothed red backed vole—*Clethrionomys rufocanus* Sund	T N Dunayeva
Northern red backed vole—*Clethrionomys rutilus* Pall	T N Dunayeva
Steppe lemming—*Lagurus lagurus* Pall	N G Olsufyev, T N Dunayeva et al, 1950
Northern mole vole—*Ellobius talpinus* Pall	N G Milyutin and A B Guz, 1939
Norwegian lemming—*Lemmus lemmus* L	T N Dunayeva
Domestic mouse—*Mus musculus* L	S V Suvorov, A A Wolfertz and M M Voronkova, 1928
Forest mouse—*Apodemus silvaticus* L	N G Olsufyev, T N. Dunayeva et al, 1950
Yellow necked mouse—*Apodemus flavicollis* Melch	The same

(Continued)

Species	Authors and date
Asian forest mouse—*Apodemus speciosus* Temm	T N Dunayeva and N G Olsufyev, 1951
Harvest mouse—*Micromys minutus* Pall	N G Olsufyev and T N Dunayeva et al, 1950
Common hamster—*Cricetus cricetus* L	N G Olsufyev T N Dunayeva et al, 1950
Ciscaucasian golden hamster—*Mesocricetus raddei* Nehr	T N Dunayeva 1951
Transcaucasian golden hamster—*Mesocricetus brandti* Nehr	The same
Striped hamster—*Cricetulus barabensis* Pall	The same
Rat like hamster—*Cricetulus triton* Winton	The same
Grey hamster—*Cricetulus migratorius* Pall	The same
Striped hairy footed hamster—*Phodopus sungorus* Pall	The same
Russian mole rat—*Spalax microphthalmus* Guld	N G Milyutin and A B Guz 1950
Midday gerbil—*Meriones meridianus* Pall	T N Dunayeva 1951
Mongolian gerbil—*Meriones unguiculatus* Milne Edw	N A Orlova 1946
Tamarisk gerbil—*Meriones tamariscinus* Pall	G I Novikova T N Ilusina 1955
Libyan gerbil—*Meriones erythrourus* Gray	U A Mamed Zade and S A Bakayeva 1957
Northern birch mouse—*Sicista betulina* Pall	N G Olsufyev, T N Dunayeva et al, 1950
Common dormouse—*Muscardinus avellanarius* L	The same
Altai zocor—*Myospalax myospalax* Laxm	T N Dunayeva
Insectivora	
Mole—*Talpa europaeae* L	N G Olsufyev and T N Dunayeva 1950
Common shrew—*Sorex araneus* L	T N Dunayeva N G Olsufyev and I M Tsvetkova 1949
Lesser shrew—*Sorex minutus* L	N G Olsufyev and T N Dunayeva 1950
Pygmy shrew—*Sorex tscherskii* Ogn	N G Olsufyev and T N Dunayeva 1950

B Mammal Species Experimentally Referred to Group II

(susceptible but low sensitive to tularemia)

Species	Minimum infective dose (microbe cells)	Minimum complete lethal dose (microbe cells)	Authors and date
Rodents			
Field mouse—*Apodemus agrarius* Pall	0 1 1	100 mln	B V Voskresensky (1940) Y A Isakov and S P Karpov (1945) T N Dunayeva and N G Olsufyev (1952)
Black rat—*Rattus rattus* L	Not established but below 1 000	1 000 mln	T N Dunayeva 1954
Grey rat—*Rattus norvegicus* Berk	0 1 1	1 000 mln	[illegible] A [illegible] 1950
Short tailed bandicoot rat—*Nesokia indica* Gray	1	1 000 mln and more	T N Dunayeva 1954
Squirrel—*Sciurus vulgaris* L	1	1 000 mln	T N Dunayeva 1954
Chipmunk—*Eutamias sibiricus* Laxm	1	500 mln	O K Kupressova and F S Sumarokov 1956
Speckled suslik—*Citellus suslicus* Guld	Not est no less than 100	1 000 mln	N G Olsufyev et coll 1950
Large suslik—*Citellus major* Pall	Not established	Over 10 mln	T N Dunayeva 1954
Long tailed suslik—*Citellus undulatus* Pall	1	1 000 mln	N A Gaisky 1944
Small suslik—*Citellus pygmaeus* Pall	0 1 1	1 000 mln	T N Dunayeva and L A Pshenichnaya (1953) N I Makarov T P Makarova and V G Bagayeva (1955)
Yellow suslik—*Citellus fulvus* Licht	Not established	Over 10 mln	T N Dunayeva

(Continued)

Species	Minimum infective dose (microbe cells)	Minimum complete lethal dose (microbe cells)	Authors and date
Relict suslik—*Citellus relictus* Kaschkar	Not established	Over 10 mln	T N Dunayeva
Thin toed suslik—*Spermophilopsis leptodactylus* Licht	Less than 10	Over 10 mln	The same
Beaver—*Castor fiber* L	Not established below 1,000	Over 10 mln	T N Dunayeva 1953
Coypu—*Myocastor coypus* Mollina	Not established	Over 10 000	F S Cherkassky A N Mashkov and I S Sorina 1951
Forest dormouse—*Dyromys nitedula* Pall	1	10 mln	T N Dunayeva 1954
Insectivora			
Desman—*Desmana moschata* L	1 10	1,000 mln	T N Dunayeva 1954
Water shrew—*Neomys fodiens* Schreb	Not established	1 mln	T N Dunayeva N G Olsufyev and I M Tsvetkova 1959
Lesser white toothed shrew—*Crocidura suaveolens* Pall	1 10	Over 1,000	N G Olsufyev and T N Dunayeva 1951
European hedgehog—*Erinaceus europaeus* L	10	2 000 million	The same
Long-eared hedgehog—*Hemiechinus auritus* Gm	Not established below 1 000	1 000 mln	The same

C Vertebrates Experimentally Referred to Group III
(low susceptible and practically insensitive)

Species	Authors and date
Weasel — *Mustela nivalis* L	N G Olsufyev and T N Dunayeva 1951

(Continued)

Species	Authors and date
Ermine—*Mustela erminea* L	T N Dunayeva 1954
Polecat—*Mustela putorius* L	N G Olsufyev and T N Dunayeva 1951
Steppe weasel—*Mustela eversmanni* Lesson	T N Dunayeva, 1954
Racoon dog—*Nyctereutus procionoides* Gray	N G Olsufyev T N Dunayeva et al 1950
Fox—*Vulpes vulpes* L	The same
Lake frog—*Rana ridibunda*	Z I Muravyova 1958

When studying a natural focus of tularemia, it is important to determine the group of animal species hitherto unsubjected to such tests The respective experiments should be staged with freshly isolated strains of tularemic bacteria, and, if possible, on adult animals, providing them with adequate maintenance and food

The objects of the work are (a) to establish the minimum complete infective dose (DCIM) and minimum complete lethal dose (DCLM) for the given species, (b) to determine the degree of insemination in the tissues and organs of lethal cases The former values are necessary to estimate the susceptibility, and the latter—the infectional sensitivity of the given species to tularemia

For animals of group I, these two values coincide, and in subcutaneous injection comprise only one microbe cell For group II the DCIM is likewise usually 1 microbe cell, whereas the DCLM varies from 10,000 to 1,000 million cells and more, depending on species Finally, for group III, the DCIM comprises 100 and more cells, while the DCLM in a number of cases is practically indefinable, exceeding 10,000 million cells

The experiments are conducted by the standard (subcutaneous) method of infection with a two day-old culture from curdled yolk media If the animal is supposed to belong to group I, infection is carried out with doses of 1,10 and 100 cells, taking 3-5 animals per dose Group II is infected with from 1 to 1,000 million cells, group III—from 100 to 10,000 million The doses should be in decimal ratio (1, 10, 100, 1000 microbe cells, etc) or, at any rate, with a shortage of animals, every dose should be 100 times its predecessor (1, 100, 10,000, 1,000,000 cells, etc)

To find out, roughly, the group relation of the given species allocated for study, 2 or 3 specimens should be infected with a 10 cell dose Death from this dose indicates that the animal probably belongs to group I, survival permits to refer it to groups II or III This being accomplished, full scale experiments are staged on the already described pattern The organs and tissues of dead animals are used for smears in which the rate of tularemia seeding is assessed by the accepted scale of designation (see p 245) Organic changes revealed at autopsy are described and recorded A definitive proof of death from tularemia is obtained by isolation of the initial culture from the viscera In surviving animals, the blood serum is studied 20 to 25 days after infection by means of the agglutination test (for procedure see p 249) Only absolutely clear-cut reactions (3 or 4 plus) are considered of diagnostic value The discovery of tularemia antibodies in the serum, even in its lowest dilutions (1 5 to 1 10) may be accepted as proof of infection The absence of antibodies indicates that the animal has not been infected

Transmission and maintenance of the infection by blood sucking arthropods The role of blood sucking arthropods as vectors of the tularemia pathogen, has been the object of detailed study in the U S S R The number of species experimentally shown to be able to transmit or maintain the agent, approaches 54 This number includes 8 species of *Ixodidae*, 2 of *Argasidae*, 9 of *Gamasidae*, 3 species of lice, 9 of fleas, 1 bug, 13 *Tabanidae*, 8 mosquitoes and 1 stable fly (Table 4)

Table 4

Blood Sucking Arthropods of the U S S R Experimentally Proved Able to Transmit or Maintain the Tularemia Pathogen

Species	Transmission	Maintenance	Authors and date
Ixodidae			
Ixodes apronophorus P Sch	+	+	D A Golov 1934
Ixodes ricinus L	+	+	N G Olsufyev 1943
Ixodes laguri Olen	+	+	V P Bozhenko and S F Shevchenko
Rhipicephalus rossicus Jak	+	+	Y F Shatas and N A Bystrova 1954
Haemaphysalis otophila P Sch	+	+	V G Petrov
Haemaphysalis concinna C L Koch	+	+	V G Petrov
Dermacentor marginatus Sulz	+	+	D A Golov and V N Fyodorov 1931
Dermacentor pictus Herm	+	+	N G Olsufyev and L N Tolstukhina 1941
Argasidae			
Ornithodorus lahorensis Neum	+	+	L S Yershova 1959
Ornithodorus papillipes Bir		+	N G Olsufyev
Gamasidae			
Laelaps hilaris C L Koch (=L festinus)		+	N G Olsufyev 1940
Haemogamasus nidi Mich		+	The same
Haemolaelaps sp		+	The same
Macrolaelaps muris Ljungh (=*Laelaps echidninus*)	+	+	N K Grzhebina 1939
Haemolaelaps glasgowi Ew (=*H mohrae*)	+		I N Nelzina and V P Romanova, 1951
Hirstionyssus musculi Johnst	+	+	L N Nelzina et coll , 1957
Hirstionyssus isabellinus Oudms	+	+	The same
Hirstionyssus criceti Sulz	+	+	The same
Ornithonyssus bacoti Hirst	+	+	Hopla C I 1951
Lice			
Hoplopleura sp (water rat louse)	+		D A Golov, 19[illegible]
Hoplopleura acantopus Burm	+		N G Olsufyev, [illegible]

17*

(Continued)

Species	Transmission	Maintenance	Authors and date
Polyplax spinulosa Denny	+		S P Karpov and V M Popov, 1936
Fleas			
Ceratophyllus walkeri Roths	+		D A Golov and V L Tiflov 1934
Ceratophyllus fasciatus Bosc	+	+	L A Dudolkina, 1951
Ceratophyllus penicilliger Grube		+	O N Sazonova, 1953
Neopsylla setosa Wagn		+	D A Golov and V E Tiflov, 1934
Ctenophthalmus assimilis Tasch	+	+	N G Olsufyev and L N Tolstukhina, 1941
Ctenophthalmus orientalis Wagn	+	+	A A Volfertz and S A Kolpakova 1946
Amphipsylla rossica Wagn		+	O N Sazonova, 1953
Leptopsylla segnis Schon	+	+	L A Dudolkina 1951
Xenopsylla cheopis Roths	+	+	The same
Bugs			
Cimex lectularius L	+	+	V P Bozhenko, 1936
Diptera **Tabanidae**			
Chrysops relictus Mg	+	+	V P Bozhenko 1941
Chrysops caecutiens L	+	+	The same
Chrysops flavipes Mg	+	+	The same
Tabanus bromius L	+	+	N G Olsufyev and D A Golov, 1936
Tabanus autumnalis L	+	+	The same
Tabanus solstitialis Schin	+	+	The same
Tabanus turkestanus Sz	+	+	The same
Tabanus erberi Br	+	+	The same
Tabanus peculiaris Sz	+	+	The same
Tabanus flavoguttatus Sz	+	+	The same
Tabanus pulchellus Lw	+	+	The same
Tabanus agrestis Wd	+	+	N G Olsufyev and D A Golov, 1940
Chrysozona turkestanica Krb	+	+	The same, 1936

(Continued)

Species	Trans-mission	Main-tenance	Authors and date
Mosquitoes			
Anopheles maculipennis Mgn.	+	+	V. N. Fyodorov and F N Sivolobov, 1935, V P Romanova and M I Danilova, 1939
Anopheles hyrcanus Pall	+	+	N G Olsufyev and D A. Golov, 1938
Aëdes vexans Mgn	+	+	The same
Aëdes caspius Pall	+	+	The same
Aedes flavescens Müll	+	+	The same
Aëdes cinereus Mgn		+	The same
Mansonia richiardii Fic	+	+	N G Olsufyev and D. A Golov, 1936
Culex modestus Fic		+	The same
Stable-Flies F			
Stomoxys calcitrans F.	+	+	P. V Somov et coll, 1937

Note The plus sign denotes positive results

Experiments have demonstrated that the cited groups of arthropods vary in their ability to transmit and maintain the causative agent of tularemia.

The specific vectors of tularemia are: *Ixodidae* and certain species of *Gamasidae*, which are obligate hematophagi, easily transmitting the infection from diseased to healthy animals. Ixodid ticks maintain the pathogen throughout their metamorphosis—from larvae to the mature stage, which is enhanced by the accelerated reproduction of tularemic bacteria in the tick's organism during suction.

Mature ticks may transmit the infection not only to rodents, but also to domestic animals (D. A. Golov, 1935). The ticks can maintain the pathogens all their life; thus, V. G. Petrov proved the maintenance of the pathogen in mature ticks *Dermacentor marginatus* during 700 days, its basic biological properties, including virulence, remaining unaffected

Owing to their longevity, *Ixodidae* serve not only as vectors but also as long term reservoirs of the infection in nature during interepizootic periods

As evident from literature, ticks transmit the pathogen of tularemia transovarially (V P Bozhenko and V. P Romanova, 1956) This, however, was not confirmed by the extremely thorough control experiments of V G Petrov (1959) on ticks *Dermacentor marginatus*, which agrees with the findings of D A Golov (1934) and other authors An extremely active vector of tularemia are rodent lice, whereas transmission through fleas and bugs is difficult, horse flies, mosquitoes and stable flies may likewise transmit the infection from diseased to healthy animals, but only mechanically

If the role of *Ixodidae*, fleas, mosquitoes, horse-flies, and stable-flies as vectors of tularemia, has been studied with sufficient detail, data on other groups of blood sucking arthropods are scarce Fuller information is necessary on the transmission and maintenance of tularemia by *Gamasidae* and *Argasidae*, and on the possibility of the same by mosquitoes, blackflies and midges

Experiments on the transmission of tularemia by blood sucking arthropods can only be staged on animal donors from group I—water rats, common voles hamsters, etc The same purpose may occasionally be served by white mice, as well as golden hamsters and steppe lemmings, which readily multiply in confinement Guinea pigs are useless as donors because of the poor development of tularemic septicemia in their bodies The animals are infected subcutaneously either with fully virulent strains freshly isolated from a focus, or a strain cultivated artificially on sensitive animals, or else dessicated under vacuum shortly after isolation

The experimental ticks or other insects may be bred in the laboratory or caught in nature The arthropods are fed on the experimental animal in groups of several dozen, hundred, or thousand specimens, depending on the size of vector and donor, the blood meal taking place towards the end of the latter's life, when its blood holds the greatest *amount of* tularemic *bacteria* (at the *stage of* septicemia)

The success of vector infection is estimated bacterioscopically, for which two or three freshly fed specimens are crushed on a slide, making a smear of blood from the stomach and staining it (after preliminary fixation with Nikiforov's mixture) by the Romanowsky Giemsa method In subsequent experiments, use is made only of series of ticks or insects replete with blood containing large quantities of tularemic bacteria (up to III or IV points) The infected arthropods are then used in tests for ability to maintain or transmit infection

In experiments on transmission the arthropods are fed on animals sensitive to tularemia (group I) The best recipients are guinea pigs or white mice, as well as various wild rodents likewise belonging to group I (hamsters, voles, etc) Pathogen carrying ticks and insects are fed pending different intervals after infection by blood meals on donor animals, viz, from several hours or days to several months, depending on the rate of blood digestion in the given arthropod species and its capacity for repeated blood meals Another procedure used is interrupted feeding, when the arthropod is removed from the donor before engorgement and is directly (or after several hours) fed to repletion on the recipient

Infected arthropods are fed on recipient animals individually or in small groups Blood meals may be effected by letting the insects freely alight on the animal, or placing them on definite areas of the latter's bodies under small glass or plastic cylinders or special adhesive bags A cylinder is glued to the shaved skin area with Mendeleyev's putty, and a bag—with celloidin dissolved in a mixture of alcohol and ether

Each animal is used for feeding infected arthropods only once, after which it is kept under observation for 15 to 20 days Dead animals are investigated for tularemia, confirming the diagnosis by isolation of the initial culture

The time of maintenance of the pathogen in the vector organism is mostly estimated by subcutaneous injection of a suspension of the arthropod tissues into an animal sensitive to tularemia, usually a white mouse The suspension is prepared in saline

The amount of tularemia microbes in the vector's body may be estimated by "titration" on white mice Five or ten specimens of arthropods subject to investigation are thoroughly crushed in a mortar After adding 2 5 or 5 ml respectively of saline solution, a homogeneous suspension is obtained, every 0 5 ml of which contains the crushed tissues of one arthropod After that the suspension is diluted 10, 100, etc, times, for which 0 5 ml of the latter is mixed with 4 5 ml of saline, and so on, the operation each time being performed with a fresh pipette The result is a number of suspensions, 0 5 ml of which contain 1/10, 1/100, etc., of the arthropod tissues These are further injected into white mice, using 0 5 ml per injection and 3 mice per suspension The value of the minimum dilution causing lethal tularemia in all 3 mice indicates the quantity of doses lethal for mice contained in the body of one arthropod, and since the DCLM of a virulent tularemic culture for white mice comprises 1 microbe cell, the said value simultaneously indicates the amount of tularemia microbes contained in one arthropod Thus, if the death of all three mice was caused by a minimum dilution of 1 100,000, one arthropod should have contained an average of 100,000 tularemia microbes The same procedure may be used for determining the amount of bacteria in individual organs of vertebrates, invertebrates, water, etc

Other routes of transmission In contrast to a number of other diseases with natural foci, such as the plague, rickettsioses, tick-borne encephalitis, etc , tularemia may be transmitted from diseased to healthy animals not only through blood sucking arthropods, but by other routes as well In the acute phase of the disease, tularemia infected rodents, especially those of group I, discharge the pathogen with the urine and faeces, thereby contaminating the environments Experiments have shown that if rodents dying of tularemia fall into water, their carcasses, *especially when damaged, may infect the reservoir* (T N Dunayeva, 1959) At temperatures from 4 to 6° C, the pathogen may retain its viability in water and on the surface of fodder (grain, straw) for as long as 4 months

In nature, small rodents often contract the disease by eating the carcasses of other rodents which have died of tularemia

According to S P Karpov (1955) and A A Selesnyova (1950, 1955), *Mollusca, Trichoptera* and other invertebrates, so called hydrobionts, receive tularemia microbes through water, in their own turn becoming sources of infection, liable to infect fresh-water reservoirs

Experiments by T N Dunayeva (1959) on water rats and other rodents, demonstrated the possibility of rodents contracting tularemia at short term immersion in infected water Infection occurred at a minimum concentration of 100 microbe cells per 1 ml of water

Experiments with infected food (grain) showed the latter to be a potential source of infection for common voles and domestic mice (L A Pomanskaya, 1957)

Research in natural foci of tularemia should envisage further elucidation of the routes of transmission through environment, especially water It is necessary to clarify the possibilities of infection through water for a number of *amphibiotic vertebrate species, e g ,* the musk rat, certain voles, etc Study should likewise be continued on the role of hydrobionts in the infection of water reservoirs, elucidating the concentrations of microbes in water at which hydrobionts may be infected, the occurrence of microbe reproduction in the latter, the temperatures at which this is possible, etc , etc

These studies may be accomplished by the techniques employed in investigating other aspects of the epizootology of tularemia In particular, "titration" on white mice may be applied for determining the amount of microbes in hydrobionts, ambient water, etc. (see p 264)

Attention should be drawn to the possibility of transmission by droplets as well as dust

Epidemiology

As demonstrated by many Soviet authors, the main sources of tularemia infection for man in the U S S R are common voles, water rats, domestic mice, hares, and, partly, musk rats and hamsters Contagion in man results either from contact with diseased and dead rodents, or from the bites of infected blood sucking arthropods (mosquitoes, horse flies, ticks), or, further, from handling water, food, straw and other substrate contaminated by rodents infected with tularemia Cases are known when personnel at meat-processing plants contracted the disease when slaughtering cattle infested with tularemia carrying ticks As regards the concrete mechanisms of contagion, man may be infected through contact, ingestion, aspiration and inoculation (arthropod bites)

Tularemia may occur in the form of sporadic cases or epidemic outbreaks of varying intensity The disease affects agricultural workers, hunters, fishermen, housewives, schoolchildren, etc , in other words, any occupational categories and age groups, including infants and old people regardless of season The specific assortment, population and ecology of rodents—the principal reservoirs—and blood-sucking arthropod vectors, as well as the various supplementary routes of transmission—all these characteristics of a natural focus have their effect on the conditions of human contagion The said characteristics, however, are in turn influenced by the social factor, i e , the concrete forms of human activity within the focus, in the course of which man comes into contact with rodents, bloodsucking arthropods or substrata (water, grain, fodder, etc) contaminated by diseased animals The conditions of contagion and especially the infection rate are also affected by preventive measures and the percentage of non immune population.

According to origin, outbreaks and solitary cases of tularemia may be classified as follows vector-borne (i e , disseminated through vectors), trappers' (from water rats and musk rats), water-borne (contracted through drinking or washing with infected water from wells or natural reservoirs), agricultural (contracted at overhaul of rodent-infected hay, contaminated grain and vegetables and transportation of hay, etc), domestic, game borne (received through hares, etc), food borne (developed through the use of milk, bread, etc , infected by rodents), industrial (contracted at plants while processing infected agricultural products) and so forth In various types of foci, the conditions of human contagion may differ, due to which epidemiological data should form an indispensable part of the description of a given focus

The primary stage of research on the epidemiological status of the focus under survey, constitutes careful accumulation of long-term data on previous outbreaks and miscellaneous cases, desirably, since the earliest known

When collected through the local public health service, morbidity data may not always correctly reflect the actual rate observed in the past, since part of the patients may have remained unregistered To verify this, resort should be taken to selective tests of the population by the percutaneous tularin technique This method permits sufficiently accurate recording of individuals earlier afflicted with the disease, since they retain the allergic skin reaction to tularin for many years Interrogation will help to determine the approximate time of the disease, it being borne in mind, however, that the persons questioned are often unaware of having had the disease Surveys should cover only the population never inoculated against tularemia

The collected morbidity data are analysed statistically and epidemiologically in the following sequence

(a) Dynamics of tularemia morbidity for the entire period covered by the available data Distribution of morbidity over the area (map), among individual settlements (diagrams), and its level for different years and seasons (graphs) and likewise in relation to conditions of contagion, occupation, age, sex, etc (graphs and tables) Morbidity indices per 10,000 inhabitants, per settlement, etc Types of tularemia outbreaks with detailed descriptions of each *sources and routes of infection, clinical forms of disease* (according to localisation of primary lesions)

(b) Epizootologic and epidemiologic factors conducive *to the incidence of tularemia Dynamics of rodent and vec*tor populations for the years covered by epidemiological analysis Data on the incidence of the pathogen in rodents and vectors Features of human economic activity involving exposure hunting of water rats, hares and other animals, haymaking and other agricultural occupations in the natural foci of tularemia Characteristics of agrotechnical procedures, promptness and efficiency of harvesting etc

(c) Effects of prophylaxis on morbidity dynamics Vaccination, rodent control, etc Evaluation of the efficiency *of adopted measures*

TYPES OF NATURAL FOCI AND ELEMENTARY FOCI OF TULAREMIA

The sources of tularemia infection, the routes of its circulation in nature and the conditions of human contagion vary with the terrain, which necessitates classification of natural foci as to their biocenotic, *epizootologic and epi*demiologic features

Fig 31. Floodland swamp type tularemic focus Lake overgrown with reeds—habitat of water rats, mosquitoes and horse flies The valley of the Karatal river near Ush-Tobeh Station, south-eastern Kazakhstan.

According to A. A Maximov (1957), "A class of natural foci permanently associated with a corresponding type of terrain is defined as a category of infective focal entities homogeneous in the character, historic origin and present-day environment of their component biocenoses, and exhibiting identical epizootologic and epidemiologic manifestations."

Each type of focus has its structure, i.e., aggregate conditions (warm-blooded animals, blood-sucking arthropods, actual forms of contact between the former and latter, relations with environment, etc.) allowing the causative agent to exist (circulate) for an indefinite length of time within the focal area (N. G. Olsufyev, 1947).

As regards the territory of the U.S S R., it appears justifiable to distinguish the following 6 types of natural tularemic foci: swamp-floodland, grassland-meadowland, woodland, steppeland (ravine); cisalpine-stream type, and desert-floodland (the latter term being proposed by G. A. Kondrashkin, 1957). As evident from the list, the terms denominating focal types are not the synonyms of the geographic zones with which they are associated. The choice of denominations was governed by the concrete assortment of biotopes harbouring the infective foci. The proposed classification sets no limit for subdivision of earlier established types and introduction of new ones. Such typing *should be based on concrete data concerning the bio*cenotic, epizootological and epidemiological characteristics of a *focus*, and *not merely on its association* with a given type of terrain.

The close interdependence between the focal type and the conditions of human exposure may be confirmed by examples A most typical occurrence in grassland-meadowland foci are agricultural and winter-time water-borne outbreaks; in the swamp and floodland type—outbreaks of

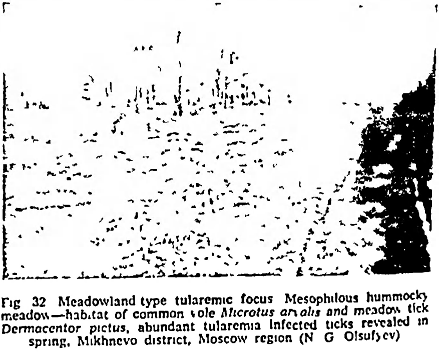

Fig 32 Meadowland type tularemic focus Mesophilous hummocky meadow—habitat of common vole *Microtus arvalis and* meadow tick *Dermacentor pictus*, abundant tularemia infected ticks revealed in spring, Mikhnevo district, Moscow region (N G Olsufyev)

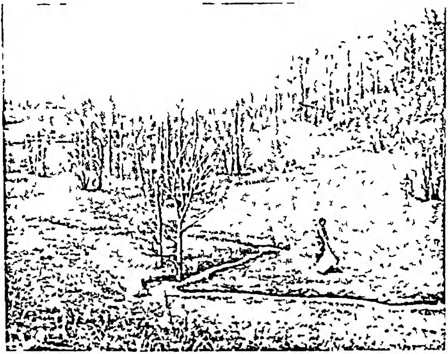

Fig 33. Cisalpine-stream-type tularemic focus Small stream with banks inhabited by water rats and ticks *Dermacentor silvarum* and *Haemaphysalis concinna* Tularemia-infected ticks revealed in spring, infected rats and water—in summer. Ticks collected on flag early in spring, Staro-Bardin district, Altai territory (N. G. Olsufyev)

the vector-borne and trappers' varieties; in cisalpine-stream foci—summer-time water-borne outbreaks, etc., etc.

At present, the swamp-floodland and grassland-meadowland types of foci are known best. The former is widespread in the European and Asian parts of the U.S.S.R., being associated with river valleys, lake shores, and swamps. The principal function in maintaining this type of foci belongs to the water rat. The grassland-meadowland type of focus, chiefly connected with the common vole, occurs mainly in the central regions of the European U.S.S.R. However, certain varieties of the swamp-floodland type, in particular the lake and swamp foci of the forest steppe zone in Western Siberia, require further investigation Less studied are the steppeland (ravine), cisalpine stream and woodland types of foci, and still poorer—the desert floodland type

Additional attention should be afforded to the natural foci of Eastern Siberia and Transcaucasia, the latter's typological features still being not clear. Little studied are the foci located in the north of the U S S R, particularly, in the lower reaches of the river Ob, and on the river Lena and its tributaries.

Classification of natural tularemic foci is based on a combination of all data accumulated during focal surveys The former should be regarded as the final stage of all research, being of major importance for substantiating the prophylactic measures to be taken in foci of different types

The focal area is subdivided into smaller sections which are called microfoci, or elementary foci, in which the infection persists with relative constancy, including interepizootic periods Elementary foci are usually associated with areas of relatively stable rodent and tick populations, mostly being polyhostal and polyvectoral

The stability of an elementary focus is to a considerable extent dependent on the longevity of ixodid ticks maintaining the pathogen during interepizootic periods (N G Olsufyev) A supplementary part in stabilising the foci is apparently played by certain species of *Gamasidae* (E. N Nelzina) The stability of natural foci of tularemia is demonstrable by the following example In Mikhnevo district of Moscow region the existence of tularemic infection in the rodent or tick population was observed continuously for 15 years (from 1938 to 1953) on an area not more than 5 kilometres broad (N G Olsufyev et coll) The discovery of elementary foci facilitates the sanitation of the focus as a whole, since prophylactic measures may be effected not on the whole area at once, but only part by part

The detection of elementary foci will be enhanced by dividing the focal area into sections with uniform ecology and terrain The procedure is as follows (1) According to features of terrain, the investigated area is sectionalised into primary geomorphological and geobotanical districts, priority being afforded to the hydrographic network and types of water reservoirs (2) According to the specific aspects (quantitative interrelationships) of the basic reservoirs and vectors and their population dynamics, districts with closely similar faunistic communities and synchronous fluctuations of rodent and vector populations are revealed (3) On the basis of epizootological characteristics, a list is made of districts harbouring epizootics with identical frequency, intensity, dissemination, seasonal occurrence, and specific assortment of animals involved, and with an equal numerical incidence of infected blood sucking arthropods (4) On the basis of economic data, districts of equal development are defined, note being made of population density, principal occupations, etc

All the above are mapped, and pending analysis of aggregate features, the territorial divisions are classed into (a) non enzootic, (b) enzootic with rare manifestations of epizootics of varying intensity, and (c) permanently enzootic areas

Division into districts according to features of terrain was successfully applied in locating elementary foci of tularemia in a survey of a floodland swamp type focus in the north of the Volga Akhtuba floodlands (N G Olsufyev, V V Kucheruk et al, 1958)

NOSOGEOGRAPHY OF TULAREMIA

As is true of all diseases with natural foci, the distribution of tularemia is primarily dependent on natural factors However, the economic activity of man also has its effect on the geography of the disease, restricting, or, sometimes, extending the boundaries of its natural foci During the almost fifty years after the discovery and description of the tularemia pathogen, incidence of the disease has been recorded exclusively in the northern hemisphere In the Americas, tularemia has been confronted in all states but one of the U S A, as also in Alaska, Canada, Mexico and Venezuela In Europe, tularemia has been diagnosed in France, Belgium, the Netherlands, Switzerland, Italy, Austria, West Germany, the German Democratic Republic, Sweden, Norway, Finland, Poland, Czechoslovakia, Rumania, Hungary, Yugoslavia, Greece, in Asia—in Turkey and Japan The global distribution of tularemia has not been fully clarified This refers to a number of continents, including Europe, where its discovery is to be expected *in several more countries, viz, Bulgaria, Albania*, Spain, and, possibly, Denmark, and among Asian countries—Iran, the Chinese People's Republic, the Korean People's Democratic Republic, and, probably, India

Since its discovery in the U S S R. (1926), an increasing amount of data have been accrued on the prevalence of natural foci of tularemia in the country. By now such foci have been revealed in both the European and Asian parts of the Soviet Union (see Fig 34) A number of regions have not been explored

The task for the future is to organise surveys of blank areas, i e, regions and districts where tularemia has not been recorded, with a view to checking the real extent to which they are free of natural foci of the disease Prompt detection of the latter is extremely important for the timely institution of prophylaxis

Detection of latent natural foci may be effected by the following procedures

(a) bacteriological study of *Ixodidae* collected in large quantities in spring from domestic animals (cattle, sheep) in various parts of the area under survey, this concerns only tick species whose larval and nymphal stages parasitise rodents (e g, species of the genus *Dermacentor*),

(b) bacteriological study of rodents, primarily, the mass

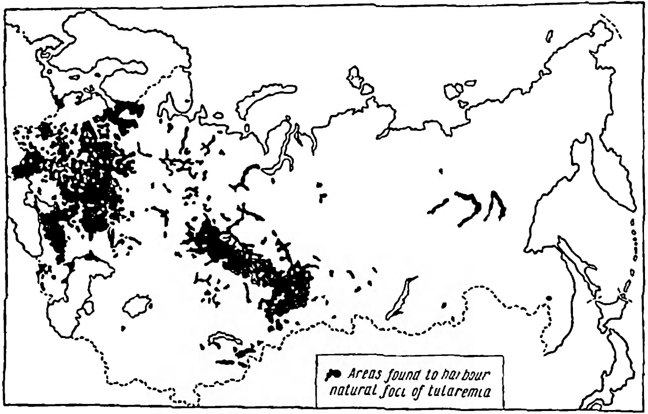

Fig. 34 Distribution of natural tularemic foci in the U.S.S.R.

spread species of group I, collected at the seasons of their most probable occurrence in the tularemia infected animal population (see p 243),

(c) selective investigation of the human population with the use of the tularin skin test, covering, primarily, persons occupationally subject to frequent contact with rodents or ticks—hunters, herdsmen, etc, permanently living in the area under survey, the survey should be restricted to people never vaccinated against tularemia,

(d) agglutination tests with the use of tularemic *diagnosticum* to be staged on the sera of cattle and sheep regularly grazing on pastures and exposed to mass tick infestment (M V Vasilyeva et coll, 1957, V L Adamovich and Y M Feldman) The test is made by volume and regarded as positive only with absolutely clear agglutination (3 or 4 plus) in serum dilutions of 1 10 and more on condition that the reaction is absent in parallel tests for brucellosis

In establishing the incidence of tularemic foci in the area under survey (i e, recognising it as enzootic in regard to the disease), serologic findings represent merely a first approach, and must be confirmed either by isolation of the pathogen from ticks or rodents or by revealing postconvalescents among the local human population

PROPHYLAXIS

At present, prophylaxis is chiefly confined to vaccination of people in the foci of the infection As generally known, the live vaccine proposed by N A Gaisky and B Y Elbert, is applied in the same way as the smallpox vaccine (B Y Elbert)

Vaccination is a reliable safeguard with any route of transmission The resulting immunity may sometimes last up to 5 or 6 years and more, which has permitted planned vaccination in enzootic regions, when urgent epidemiological indications were still absent Revaccination is made after five years The development of such a highly effective vaccine against tularemia is a splendid achievement of Soviet scientists

Since tularemia belongs to the diseases with natural foci, the use of vaccines alone cannot lead to sanitation of its foci, where the potential hazard of infection still remains Vaccination may be regarded merely as a provisional measure until all natural foci of the disease are suppressed Sanitation of natural foci of tularemia may be achieved by reducing the population of the principal reservoirs and vectors to a level precluding their mass reproduction

Soviet scientists have developed effective methods of exterminating rodents in tularemic foci (N P Naumov et coll, V V Kucheruk et al) Thus, water rat control is successfully achieved by the use of carrot bait poisoned with zinc phosphide The same poison as well as others proved highly effective against the common vole Control in this case is effected by fumigating the entrances of vole burrows In agricultural areas, control is based on thorough execution of agrotechnical operations, in particular, prompt removal of stubble, thorough ploughing in autumn and spring, timely and thorough harvesting, etc

Regular hunting has an important restrictive effect on the population of water rats, hares and other game rodents Systematic hunting in tularemic foci precludes the appearance of epizootics among game rodents, and thereby, along with vaccination, ensures the prevention of tularemia among people (N G Olsufyev, B P Borodin, V V. Kucheruk et al, 1958)

The destruction of *Ixodidae* is also of major importance for the sanitation of natural foci of tularemia Many species are amenable to control in the mature stage, when feeding on domestic animals

A highly effective measure is DDT and hexachlorane treatment of cattle, which leads to almost total extermination of the ticks (L N Pogodina, N G Olsufyev, V I Kurchatov, V G Petrov)

The ticks may also be exterminated by spraying the vegetation with DDT preparations (N N Gorchakovskaya, V G Petrov)

Apart from that, there are other measures, such as enforcement of sanitary rules and regulations at water sources, in shops, stores and dwellings, public sanitary education, etc

In the Soviet Union preventive measures against tularemia are effected by the entire public health network under the guidance of the departments of high hazard diseases of regional and district sanitary and epidemiological stations These departments plan vaccination campaigns, check up on their quality, carry out epizootologic surveys of natural foci of the disease, present forecasts regarding the population of rodents—the principal sources of tularemia infection—furnish the agricultural authorities with recommendations on rodent and tick control etc , etc

Prognostication of rodent population and planning of prophylactic measures are carried out differentially, with regard for the local types of tularemic foci Thus, for grassland meadowland foci, the basic measures should envisage prevention of the agricultural and winter time water borne outbreaks which are most typical for this class of foci for floodland swamp areas—the prevention of vector borne and game borne outbreaks for the cisapline stream type—prevention of summer time water borne outbreaks, and so on

The incidence of tularemia among the population is assumed as the basic criterion for evaluating preventive measures Estimates of the efficiency of preventive measures are made by analysing the sick rate for the current and subsequent years

If cases of the disease occur despite prevention, especially if their incidence is comparatively high, it is necessary to elucidate the reasons of the inefficiency of the adopted measures If morbidity is reduced or eliminated, it is necessary to evaluate the importance of adopted measures in achieving such results Analysis of the efficiency of these measures should be based on objective evaluation of domestic and labour conditions and variations in the latter, as well as on objective estimates of the scope and quality of the measures proper

Basically, the quality of prophylaxis and anti epidemic sanitation is assessed by the following (1) quality and efficiency of preventive vaccination, as assessed by comparing morbidity among vaccinated and non vaccinated individuals residing in equal epidemiological conditions or by confronting the sick rate for three, five or more years preceding the given campaign with the 3 5 and more years following, (2) promptness and effectiveness of rodent and vector control carried out in the focal areas with prophylactic purposes, (3) completeness and promptness of registration of tularemia patients, (4) efficiency of other preventive measures safeguarding and decontamination of water sources and food products, water rat hunting, etc

Analytic data on the effects of preventive and anti-epidemic measures are subsequently utilised in planning and carrying out combined anti-tularemia programmes

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LEISHMANIASES

The term leishmaniases presents the group denomination of a number of diseases caused by parasitic protozoa belonging to the genus *Leishmania* The vectors of these pathogens are insects of the genera *Phlebotomus* and *Sergentomyia* All diseases classed under this name are distinguished by their pathogenesis, clinical course and a number of other features, and are usually divided into two groups known as cutaneous and visceral leishmaniases

CUTANEOUS LEISHMANIASES

Synonyms Borovsky's disease, Borovsky's leishmaniasis, *Leishmania tropica, oriental sore* Pendinski (Ashkhabad, Kokand, Tashkent, Samarkand or Merv) ulcer, Lenkoran or Yelizavetpol godovic, Leishmaniosis cutanea (*Lat*), Leismaniose cutanee (Fr), cutaneous leishmaniasis (Eng), Hautleishmaniose (Germ), leishmaniasis cutanea (Sp)

General Definition

Cutaneous leishmaniasis is a transmissive endemic disease proper to countries with warm and hot climates The external manifestations are ulcerous lesions of the skin resulting from the bites of small blood sucking *Diptera*—sandflies *Phlebotomus* and *Sergentomyia*

Distribution

In Europe Italy, France, Spain, Greece, Yugoslavia, Bulgaria, Albania, Transcaucasian republics of the U S S R

In Asia India, Arabian peninsula, Afghanistan, Indo-China, Philippines, Ceylon, Iran, Iraq, Israel, Turkey, Syria, Jordan, Yemen, Soviet Central Asian republics

In Africa Algiers, Tunis, Libya, Morocco, Egypt, Sudan Congo, Angola, Ethiopia, valleys of the rivers Niger, Shari, and others

In South and Central America Brazil, Paraguay, Mexico, Argentina, Uruguay, Columbia, Bolivia Peru, Panama, Yucatan peninsula, Guiana, Equador, Venezuela

History The earliest descriptions of the disease were made by the British physician Pocock in 1745 in Syria The clinical features of the disease were outlined by bros Russel in 1756 In 1833 Gillhou delivered before the Paris Academy of Sciences a thesis on what he called the "Aleppo boil". Later, the same disease was examined by numerous other investigators and physicians

The Russian military doctors N Arend (1862), F Finkelstein (1886), L Heidenreich (1888), A Kolpakov (1901) described the clinical picture of the disease together with some of its prominent epidemics In 1898 G F Borovsky, an army doctor, discovered and described in some detail the causative agent of cutaneous leishmaniasis, without, however, stating its systematic relationships and name The Russian physician's discovery was confirmed by K Shulgin (1902) G F Borovsky's publication remained unknown abroad so that priority in the discovery was wrongly ascribed to J H Wright, a Boston physician who revealed the pathogen in the content of an ulcer in a child immigrant from Armenia In 1900 Leishman and in 1903 Donovan isolated the causative agent of visceral leishmaniasis Issuing from these data, R Ross in 1903 referred the infective agent of cutaneous leishmaniasis to a new genus which he called *Leishmania* after the British military doctor.

Academician E N Pavlovsky (1924-1927) re established the priority of the Russian scientist in the discovery of the pathogen of this disease Subsequently, the work of G F Borovsky was published by the British scientist C Hoare in London in the *Annals of the Royal Society of Medicine*

Among the Russian contributors to the development of the theory of leishmaniases in the republics of Central Asia, it is necessary to mention the works of E I Martsinovsky, V L Yakimov, Y N Pavlovsky, N I Khodukin I I Gitelson, N I Latyshev and A P Kryukova P P Perfilyev, Y P Vlasov, P A Petrishcheva, L M Isayev A S Artamonov, P V Kozhevnikov, N F Rodyakin L F Burova, I S Kassirsky, V P Petrov, M S Sofiev, F I Shevchenko, and many others A large number of other workers, such as S P Kandelaki, A A Mirzayan P P Popov, G M Maruashvili, are known for their research on leishmaniases in the Transcaucasia

New directions in the study of cutaneous leishmaniasis were initiated by the works of Y P Vlasov (1929 1937) and P A Petrishcheva (1930 1957) For the first time in world science, these authors proved that *sandflies*—vectors of the disease—inhabit, oviposit and develop in the burrows and lairs of wild animals The works of these authors paved the way for the discovery of the reservoirs Investigations in the field were prompted by numerous facts of mass infection with cutaneous leishmaniasis among new arrivals in unpopulated desert and semi desert areas *For the first time, the incidence of* cutaneous leishmaniasis in nature was brilliantly demonstrated by the works of expeditions under N I Latyshev

Individual Forms of Cutaneous Leishmaniasis

As proposed by P V Kozhevnikov, two types *of* cutaneous leishmaniasis are distinguished in the U S S R , the first an acutely necrotic zoonosis, known as desert or rural leishmaniasis or Pendinski ulcer, and the second, an anthroponosis with delayed ulceration known as the urban type

Zoonose Type Cutaneous Leishmaniasis

The causative agent is *Leishmania tropica major* (V L Yakimov, 1915) The incubation period is short (1 to 4 weeks) The disease develops rapidly, beginning with the appearance of an infiltrate Ulceration starts after 1 to 3 weeks, the ulcers occurring on the lower extremities more often than on the face The duration of the disease is from three to six months Severe mass outbreaks are not uncommon The reservoirs are wild rodents inhabiting the desert The disease is prevalent in rural localities suburbs and deserts *The first* extensive *outbreaks on* record took place in 1885 among Russian troops stationed in the Murgab district, afflicting up to 85 per cent of personnel in diverse detachments Considerable outbreaks were noted among French troops during the invasion of Algeria, especially in the Hafsah and Biskra oases Physicians often named the ulcers after the localities where cases were recorded

Urban Cutaneous Leishmaniasis

Causative agent—*Leishmania tropica minor* (V L Yakimov, 1915) *Incubation period prolonged (from two to* six months and up to one, two or more years) The ulcer develops slowly, three to six and more months passing until the small, hardly visible brownish swelling shows signs of ulceration Usually, ulcers form on the face, less often on the legs On healing, the ulcer leaves a scar Mass epidemic outbreaks are rare

Among animals, the red tailed gerbil and short tailed bandicoot rat are known to be susceptible to this type of pathogen (E M Belova, 1955) Newer works on animal susceptibility have not been published Several authors obtained negative results with old animals which died prior to the end of incubation It is safe to assume, however, that the list of animals susceptible to this form will increase with the number of properly staged experiments

The principal reservoir of the infection is considered to be the human patient The persistent ulcers are subject to frequent contact with sandflies, which after a suction become pathogen carriers and may infect healthy people For this reason the variety of leishmaniasis producing delayed ulceration is classed as an anthroponosis The pathogen circulates through the chain patient→ sandfly→ healthy individual

The possibility of diseased dogs acting as reservoirs cannot be ruled out, considering the frequent coincidence of cases in the former and man In experiments staged by V P Kozhevnikov, N V Dobrotvorskaya, A N Sokolova and A A Shakhova (1937), white mice proved low sensitive to urban type strains of leishmanias, and highly sensitive to those of the zoonose or desert type The principal vectors of the disease are considered to be *Phlebotomus sergenti* and *Ph papatasii*, which are both prevalent in the foci of infection The same species displayed marked susceptibility to infection with pathogen strains of the urban type (E M Belova, 1955)

Post convalescent immunity is fairly stable, and may likewise be produced by inoculation with live cultures of zoonose and urban strains Injection of live cultures of the zoonose type imparts immunity to both forms (N F Rodyakin, 1955)

Therapeutic measures are elaborated insufficiently The four to five per cent solutions of acrichine used in the U S S R have brought some success The efficiency of acrichine therapy depends on promptness of application Primary papules may be cured by intracutaneous injection of acrichine in the first one to three months after appearance

American Cutaneous Leishmaniasis

Synonyms espundia, mucocutaneous leishmaniasis, Brazilian cutaneous leishmaniasis, buba brasiliana, Leishmaniosis americana, forest yaws, leshmaniose forestiere americaine

This type of leishmaniasis is prevalent in the humid forests of Central and South America The infection is most widespread among lumbermen, affecting the mucous membranes of the nose, pharynx, larynx, and mouth, the lesions extending to the cartilage and bones The disease may continue for two, three and more years, leading to severe exhaustion and death The infection doubtlessly belongs to the class of diseases with natural foci, as interpreted by the theory of Y N Pavlovsky The reservoirs are apparently certain species of wild forest mammals, the vector being sandflies In urban localities it is rare

Prevention measures are not elaborated S Pessoa (1958) considers that vaccination could be an effective safeguard for lumbermen

Sudanese cutaneous leishmaniasis Synonym nodular cutaneous leishmaniasis Suspected causative agent—*Leishmania nilotica* Brumpt, 1913

The independence of the species is disputed

The external manifestations present nodules which commonly do not ulcerate Research has been inadequate Distribution Sudan and Egypt

Causative Agents

The causative agents are *Leishmania*, a genus of the family *Trypanosomidae*, order *Protomonadida*, class *Flagellatae*, phylum *Protozoa* (Fig 35)

During their life cycle, *Leishmania* develop two forms, the leishmanial (in vertebrate hosts) (Figs 36 and 37) and leptomonad (in the intestine of *Phlebotomi* and in culture) (Figs 38 and 39)

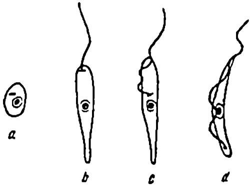

Fig 35 Systematic status of leishmanias in fam *Trypanosomidae*
a—leishmania, b—leptomonad, c—crithidia, d—trypanosome

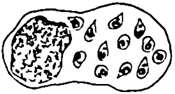

Fig 36 Position of leishmanias in cell

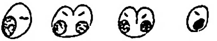

Fig 37 Reproduction of leishmanial forms

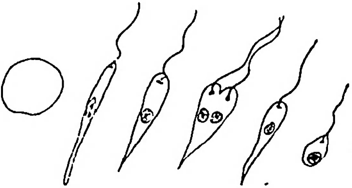

Fig 38 Leptomonad forms of *leishmanias*

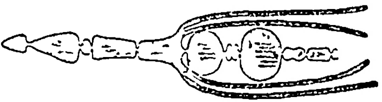

Fig 39 Position of leishmanias in sandfly intestine (M A Shoshina)

The genus *Leishmania* includes the following species (1) group *tropica*. *L tropica* with two subspecies (see further), *L brasiliensis* with several subspecies, (2) group *donovani*, including *L donovani*, *L infantum*, *L donovani* var *archibaldi*, *L d* var *chagasi* The species occurring in the U S S R are *L tropica* (with two subspecies) and *L donovani*

Basic Specific Characteristics

Leishmania tropica Wright, 1903, is the causative agent of cutaneous leishmaniasis By its biology and morphology (as noted by V L Yakimov in 1913 1915) it may be divided into two subspecies

(a) *L tropica major* Yakimoff, 1915—the infective agent of the zoonose (desert or rural) type of cutaneous leishmaniasis The parasite is 5 5×4μ in size, oval in shape The protoplasm frequently contains vacuoles On fixed and stained smears, the nucleus appears to be of loose structure In white mice causes lesions of the skin Subcutaneous injection frequently results in the development of generalised infection In nature occurs in gerbils, thin toed susliks and hedgehogs Lesions develop on the ears eyes and nose of animals

(b) *L tropica minor* Yakimoff, 1915—the causative agent of urban cutaneous leishmaniasis Size 4×3 1 μ shape—elongate oval Protoplasm homogeneous, nucleus compact White mice not susceptible The disease is an anthroponosis

L brasiliensis Vianna, 1911—causative agent of South American cutaneous leishmaniasis At present, investigators of South American cutaneous leishmaniasis distinguish three species of the pathogen (see Floch, 1957) The group tropica should apparently include a whole series of other subspecific categories viz , different geographic and ecologic races of leishmanias

Leishmania donovani Laveran et Mesnil, 1903, is morphologically similar to *L tropica major* *L donovani* affects the viscera, i e , the liver, spleen, marrow, etc The parasite reproduces in the protoplasm of Kupffer's cells, macrophagues and other phagocytes Here it at times appears in enormous quantities, destroying the cells and penetrating into fresh ones Isolated specimens occur in the blood plasm and leukocytes In smears and thick films, however, they are observed seldom Occasionally, the presence of leishmanias may be detected by inoculation of blood into different media (see further)

In various parts of the globe, the reservoirs of *L donovani* are man (India), dogs (Mediterranean area, Central Asia), or wild animals Accordingly, the anthroponose and zoonose types of visceral leishmaniasis are distinguished

Apart from the animals already mentioned, white mice, hamsters, susliks, cats, apes, and certain others also proved susceptible to *L donovani* In some of them the disease becomes generalised However, experimental infection of animals is not always successful As in cutaneous leishmaniasis, the species *L donovani* may be divided into several smaller independent categories, or geographic races Some authors have even classed the latter as independent species, such as *L infantum* Nicolle, 1908 (the causative agent of Mediterranean visceral leishmaniasis) *L donovani chagasi* Cuhna et Chagas, 1937 (the pathogen of South American visceral leishmaniasis), etc As regards the independence of *L canis* Nicolle, 1908 (*L tropica* v *canina* Yakimoff, 1915)—the causative agent of canine leishmaniasis this species should be referred to the synonyms of *L donovani*

Morphology of Leishmanial Forms

In the bodies of mammals, including man, these parasites occur only as non flagellate forms

The parasites usually reside in the protoplasm of various phagocytic cells of the skin or mucous membranes (in cutaneous leishmaniasis), and the liver, spleen, marrow, etc (as in visceral leishmaniasis) In the blood cells they occur in small quantities and far from regularly On destruction of the cell protoplasm, the parasites penetrate into the interstice spaces where they lie in free order Their shape is oval or round, size 2 to 6× 2 to 3μ (see above) On the surface, the parasite is covered with a thin membrane called the pellicle

The protoplasm is more or less homogeneous and includes vacuoli The Romanowsky Giemsa stain produces a bluish colouring The nucleus is located in the centre, being round in shape and 1 5×2 5μ in size In dry stained preparations its shape often appears to be irregular, which is due to fixation and desiccation When fixed by the wet method, the nucleus shows a typical bubble like structure The Romanowsky Giemsa stain produces a ruby red colour A highly typical feature of leishmanias is the presence of the *blepharoplast*, which is shaped as a short stick with rounded ends The *blepharoplast* usually stains deeper than the nucleus (see Fig 35)

Reproduction takes place by means of binary longitudinal fission At first, the *blepharoplast* increases in length, then it divides, which is followed by fission of the nucleus and, finally, the entire body (Fig 37) This process develops in the protoplasm of the reticulo endothelial cells Leishmanial fission is presented in Fig 37 The parasites, closely packed in a single cell of the host, may produce an impression of multiple fission The schizogonic process described by certain authors is apparently the result of an error By multiplying and filling the protoplasm, the parasites destroy the host cell, passing into the interstice and invading other adjoining cells

Development of Leishmanias in the Vector

The first to discover leptomonads in the intestine of *Phlebotomus* in 1914 was C. Wenyon, working at the time in Aleppo. However, the idea of the importance of these insects in the spread of leishmaniases was expressed earlier by Pressat (1905) and bros. Ed. and Et. Sergent. Experimental infection of man with leptomonads from *Phlebotomus* was effected by bros. Sergent, Donatien et al., in 1921.

Phlebotomus attacks the open parts of the body (the nose and lips of dogs, ears, nose and eyelids of rodents), and, piercing the skin with their suction organs, pump out not only the blood, but the tissue fluid as well. As a result, solitary leishmanial forms or the host's phagocytes packed with the former, enter the sandfly's alimentary tract. Within the latter, the leishmanias develop into leptomonads, which occupies approximately the first 20 hours. After a time, the leptomonads penetrate from the mid-intestine into the pharynx (Fig. 39) or mid-intestine. At sandfly bites, the leishmanias may enter the wound and infect the victim—man or susceptible animals. Apart from that, infection in cutaneous leishmaniasis takes place through penetration of leptomonads into the wound (at bites or scratching) from the sandfly's mid-intestine (M. A. Shoshina, 1953).

Morphology of Leptomonad Forms

In the intestinal channel of the vector, as well as in culture and citrate blood, leishmanial forms develop into leptomonads (Fig. 38). The latter's body is spindle-shaped *with one end tapering and the other blunt, 10 to 25μ long* and two to four μ wide. The nucleus lies in the middle of the body, the *blepharoplast* being noticeably anterior, of a shape and size roughly the same as in leishmanias. A flagellum 15 to 20μ long stems from the *blepharoplast*.

It should be borne in mind that, apart from leptomonads, closely related protozoan forms of the genera *Leptomonas* and *Herpetomonas* may likewise occur in the intestine of many insects. These, however, are not pathogenic to mammals, but are merely commensals inhabiting the intestinal lumen of certain invertebrates.

CUTANEOUS LEISHMANIASIS

Diagnosis

The causative agent of cutaneous leishmaniasis may be detected in a patient's skin at the site of the primary lesion. When investigating lesions in the early state (papules), the area concerned should be wiped with alcohol, applying a sterile syringe with its needle pointing towards the middle of the papule. The obtained sample of serous-sanguinolent fluid is used in smears prepared by the usual procedure, and is inoculated into a culture medium with antibiotics to obtain leishmanial cultures. In mature ulcers, leishmanias are impossible to detect owing to profuse bacterial flora and intensive cellular disintegration. The edge of the infiltrate or papule is squeezed by the fingers, making a small incision with a scalpel, taking care to avoid hemorrhage. With the tip of the scalpel, a sample of the sediment is taken from the walls and bottom of the incision. Occasionally, the needle is introduced into the edge of the ulcer remaining intact, pointing in the direction of the bottom. The obtained drop of liquid or scraping is used for smears and culturing.

Investigations on Wild Animals in Foci of Cutaneous Leishmaniasis

N. I. Latyshev and A. P. Kryukova (1941-1953), and a number of other workers, demonstrated that the principal host of *Leishmania tropica major*, the pathogen of zoonose type cutaneous leishmaniasis, is the large gerbil Less important in this respect are other rodents, such as the red-tailed and midday gerbils, and the thin toed suslik Leishmanias were likewise revealed in the long eared hedgehog

Experiments and observations in nature carried out by L N Yeliseyev and G A Sidorova (1958), and other authors, showed conclusively that in large gerbils, the leishmanial process is mostly localised in the auricle, and less frequently on the nose and eyelids The disease in these animals proceeds for more than one and a half years In gerbils, the pathologic process mostly develops on the auricle—areas almost completely free of hair and easily accessible for sandfly bites—and may be divided into the following stages (a) initial—infiltrate, (b) intermediate—abscess, and (c) final—healing The same authors find that ulcers do not develop in animals

In the initial stage, a leishmanioma two to five mm in dia forms, which is demonstrable only by palpation The skin on the ear is usually infiltrated both inside and outside the auricle On incision, a small amount of liquid is exuded The abscess is easily noticeable, having a smooth, dark brown surface At palpation, the consistence of the leishmanioma proves to be soft or pastose An incision reveals a small cavity filled with pus The associated destruction of cartilage leads to deformation of the auricle The healing stage is marked by the reduction and disintegration of the leishmanioma and disappearance of the infiltrate On the site of the tumor remains a spot of black pigment Smears prepared from the liquid or pus in all stages reveal typical leishmanias (L N Yeliseyev and G A Sidorova, 1958)

Animals are tested for leishmanias on smears of the liquid or pus obtained from the tumor by incision The latter are spread over slides then dried and treated by the usual procedure (fixation Romanowsky Giemsa staining) In some cases, the content of the abscess cavity may be scooped out for smears Lesions on other parts of the body are rare

It should be borne in mind, that the auricles of rodents not infected with leishmaniasis are the frequent feeding site of various stages of *Ixodidae* When the ticks drop off, a small depression may be seen at the site of attachment, produced by the piercing apparatus of the tick, as well as an infiltrate The latter should not be confused with the infiltrate developing in leishmaniasis (L N Yeliseyev and G A Sidorova, 1958)

Apart from smears, leishmanias may be revealed by biopsy from the affected part, the pieces obtained being cast in paraffin and used for preparing microscopic sections by the conventional histologic procedure The liquid and a suspension of scrapings from the affected parts may serve for culturing leishmanias

Natural Focality of Zoonose Type Cutaneous Leishmaniasis

As early as in 1939, by analysis of epidemiological and epizootological relationships on the materials of various expeditions, Academician Y N Pavlovsky was able to refer the desert type of cutaneous leishmaniasis to the category of diseases with natural foci

The zoonose type of cutaneous leishmaniasis exists independently from man in unpopulated deserts and semideserts, the pathogen being circulated from diseased to healthy animals through the agency of sandflies in the active period of their life During interepidemic periods, *the pathogen is maintained not in the vector, but in the* bodies of wild animals inhabiting the natural foci, and in anthropurgic foci—in human and canine ulcers Man contracts the disease when visiting focal areas during the period of sandfly activity, the latter receiving the leishmanias from wild animals. The disease affects non immune individuals

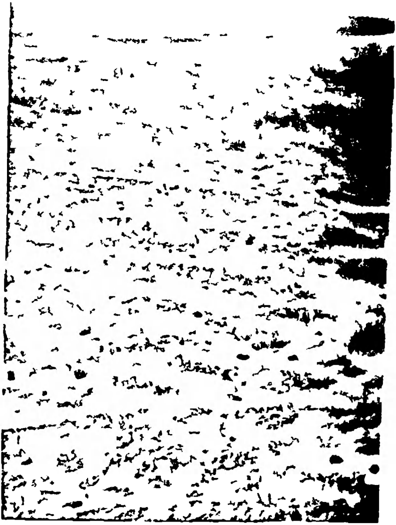

Fig 40 Colonies of large gerbils in semi-deserts of Kopet Dag foothills Natural focus of cutaneous leishmaniasis and sandfly fever (P A Petrishcheva)

The natural biotopes of cutaneous leishmaniasis may be the burrows and shelters of wild animals susceptible to the infective agent. The indispensable components of biocenoses in such burrows, apart from susceptible animals (mammals), are *sandflies as the permanent ectoparasites* of the latter, and the causative agent proper. Cutaneous leishmaniasis is an obligate transmissive disease, whose pathogen may be transmitted only through sandflies.

The completest studies of the pathogen reservoirs of cutaneous leishmaniasis have been made in the U.S.S.R. The first to approach the solution of this problem was N. I. Latyshev et coll. In 1937, while working in Turkmenia, this author found a large gerbil infected with cutaneous leishmaniasis. The said discovery was soon followed by others. In 1938, an expedition to the Murgab oasis (Turkmenia) led by N. I. Latyshev established that 67 per cent of the population of red-tailed and large gerbils were infected with cutaneous leishmaniasis. In the same year the disease was revealed in a thin-toed suslik. Subsequently, investigations on gerbils for detecting natural foci of leishmaniasis became a matter of routine.

The *large gerbil* (*Rhombomys opimus* Licht.) is a common inhabitant of the deserts and semi-deserts of Central Asia. The animal lives in colonies, whose burrows penetrate the soil in all directions, reaching a depth of one to three metres (Fig. 40). In some colonies the disease afflicts the entire population, in others the incidence is very low (one or five per cent of the animal population), despite the abundance of sandflies in the burrows. The explanation of this phenomenon has not been found. In experimental conditions, the large gerbil is highly susceptible to different modes of infection (inoculation with leptomonad cultures, transplantation of pieces of leishmanial tumors, sandfly suction).

The *red-tailed gerbil* (*Meriones erythrourus* Gray) forms considerably smaller colonies, mostly in the neighbourhood of cultivated territory, on wastelands, in villages and city suburbs. The animal is highly sensitive to infection with cutaneous leishmaniasis, although diseased specimens in nature are extremely rare. Apparently, this is explained by less frequent contact with the vector. Despite that, the red-tailed gerbil may maintain foci of cutaneous leishmaniasis (Figs. 41, 42).

Considerably less important in the epizootology of the disease is the midday gerbil (*Meriones meridianus* Pallas) and tamarisk gerbil (*Meriones tamariscinus* Pallas), despite their high susceptibility to infection in experimental conditions. In foci situated in the upper reaches of the river Amu-Darya, the percentage of diseased midday gerbils

Fig 41 Small isolated township in Kopet-Dag foothills abounding in sandflies issuing from burrows of red tailed gerbils Cases of cutaneous leishmaniasis and sandfly fever noted among population (P A Petrishcheva)

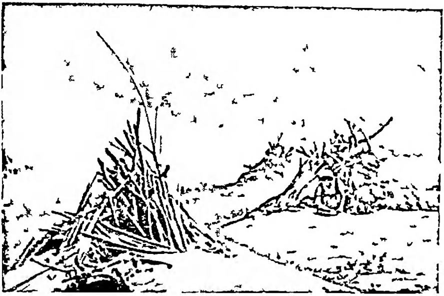

Fig 42 Crude temporary structures on mountain pastures used as storm shelters for lambs Mass infestment with sandflies and many exit holes of red tailed gerbil burrows Cases of cutaneous leishmaniasis recorded among shepherds (P A Petrishcheva)

was three seven (N I Latyshev, P V Kozhevnikov T P Povalishina, 1953) Only one diseased specimen of the tamarisk gerbil was found in the lower reaches of the same river in 1951 (P A Petrishcheva)

Readily susceptible to the infection is the thin toed suslik (*Spermophilopsis leptadactylus* Licht) Spontaneously infected specimens of the latter occur less frequently than in the case of the large gerbil Likewise highly susceptible is the short tailed bandicoot rat (*Nesokia indica*) the large Transcaspian hedgehog (*Hemiechinus albulus major*), domestic mice (*Mus musculus severtzovi* and *Mus musculus*), the small hamster (*Cricetulus migratorius*), lemmings (*Lagurus lagurus*) the rat like hamster (*Cricetulus triton*) The latter two do not occur in foci of cutaneous leishmaniasis but may be used as laboratory models for experimental studies on cutaneous leishmaniasis

Vectors

The vectors of the leishmaniasis pathogen are *Diptera* of the family *Psychodidae*, subfamily *Phlebotominae*

Over 300 species of *Phlebotominae* have been described, being distributed in all warm parts of the world The insects occur only in areas with no less than 50 warm days (the life cycle of a single generation) where the temperature does not fall below 20° C for any considerable length of time In the eastern hemisphere the insects are mostly found in arid regions, while in the western, contrariwise, they inhabit humid areas, mostly forests, subtropical and tropical regions

In the U S S R sandflies are spread through Turkmenia, Uzbekistan, Tajikistan, southern regions of Kirghizia and Kazakhstan, the republics of the Transcaucasia, the Crimea, southern Ukraine, and Moldavia The species most typical for the U S S R are *Ph papatasii* Scop , *Ph sergenti* Parrot, *Ph caucasicus* Mrz , *Ph chinensis* Newst Almost all the species encountered in the fauna of the U S S R may occur in abundance in large urban and rural settlements, as well as on uncultivated terrain in nature, where their entire life cycle is connected with wild animals

According to the new classification (O Theodor, 1948) the sandflies of the U S S R are referred to two genera *Phlebotomus* and *Sergentomyia* The former includes four subgenera (1) subgenus *Phlebotomus* with one species *Ph papatasii* Scop , (2) subgenus *Paraphlebotomus* *Ph sergenti* Parrot, *Ph caucasicus* Marzinovsckyi, *Ph mongolensis Sinton*, *Ph alexandri* Sinton, *Ph andrejevi* Shakirzjanova, (3) subgenus *Laroussius* *Ph major annandale*, *Ph perniciosus* Newstead, *Ph kandelakii* Schurenkowa, *Ph smirnovi* Perfiliev, *Ph keshishiani* Schurenkowa *Ph perfilievi* Parrot, *Ph perfilievi* var *transcaucasicus* Perfiliev *Ph tobbi* Adler &Theodor, *Ph wenyoni* Adler &Theodor, (4) subgenus *Adlerius* *Ph chinensis* Newstead The

Fig 43 Sandfly infested cave in Kopet Dag foothills near Bagir (Turkmenia) leptomonad infected sandflies revealed (P A Petrishcheva)

genus *Sergentomyia* includes two subgenera (1) subgenus *Sergentomyia* with the species *Ph arpaklensis* Perfiliev, *Ph graekovi* Chodukin, *Ph sogdiana* Parrot, *Ph sumbarica* Perfiliev, *Ph pavlovskyi* Perfiliev, *Ph minutus* Rondani, *Ph squamipleuris* Newstead, (2) subgenus *Sintonius* with one species *Ph clydei* Sinton

Of these, seven species have been described by Soviet authors Sandflies are widespread in undeveloped nature throughout a variety of zones in the subtropical regions of the Soviet Union, including all types of torrid deserts, semi deserts and steppes, woodland and scrub, the banks of rivers, irrigation canals, springs, etc A rich sandfly fauna has been discovered in the alpine regions of the republics of Central Asia (Figs 43-45)

Fig 44 Crude structure used as night shelter for goats in Kopet Dag mountains abounding in sandflies coming from burrows for blood meals (P A Petrishcheva)

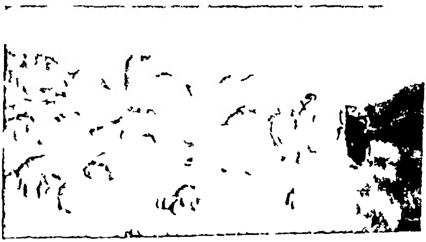

Fig 45 Daytime shelters of sandflies under scattered boulders in *unpopulated mountains* south western Kopet Dag (P A Petrishcheva)

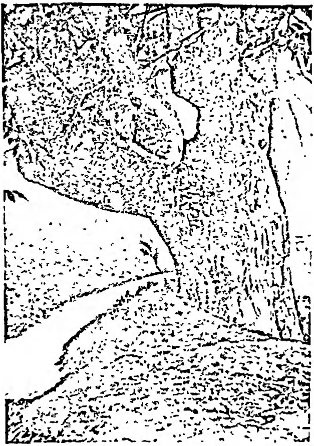

Fig. 46. Sandfly inhabited hollow in old walnut-tree (P. A. Petrishcheva).

At present more than 300 species of animals and birds have been discovered, whose nests, burrows, lairs and shelters are inhabited by sandflies. The greatest abundance of sandflies occurs in the lairs and burrows of large animals such as the fox, wolf, jackal, porcupine, large gerbil *(Rhombomys opimus* Licht.), etc. Mass reproduction of sandflies is possible in caves and grottoes inhabited by bats, lizards and other animals (Figs. 46-48).

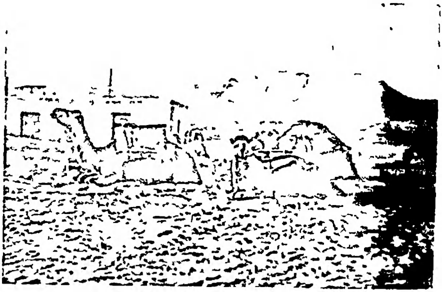

Fig 47. Second year of cultivation in small area of Central Kara-Kums Recently constructed homes infested by sandflies Human cases of cutaneous leishmaniasis on record (P. A Petrishcheva).

Mass hatching of *Phlebotominae*, testified by findings of larvae, pupae, and the occurrence of newly-fledged specimens, was recorded in the following places: (1) in settlements—in wall cracks and burrows of rodents, hedgehogs, and tortoises, in damaged wall bases of different buildings, in the basements of dwellings and service buildings, in half-ruined tombs in cemeteries, in burrows and fissures in yards, gardens and orchards (Figs. 49-50); (2) in nature—*in the burrows of the large gerbil and short-tailed* bandicoot rat *(Nesokia indica)* where, occasionally, from 400 to 500 larvae and pupae are found per burrow; hatching may also occur in the burrows of the grey rat, caraco rat and other wild animals, (3) in birds' nests, *Phlebotominae* reproduce comparatively seldom

Among the *Phlebotominae* of the U.S S R. the following are vectors of zoonose cutaneous leishmaniasis: *Phleboto-*

mus sergenti, Ph alexandri, Ph caucasicus, Ph mongolensis, Ph papatasii, S arpaklensis, S sumbarica, S clydei, Ph kandelakii, S graekovi Spontaneous infection was revealed in specimens of these species captured in the burrows of gerbils affected with cutaneous leishmaniasis Many of the aforesaid likewise proved susceptible to experimental infection A P Kryukova (1941) working in laboratory conditions, demonstrated the transmission of cutaneous leishmaniasis during the blood meals of *Ph papatasii*

The incidence of spontaneous infection among individual sandfly species in natural foci may reach high levels, and

Fig 48 Loess cliffs in lower reaches of the Artek river Fissures and caves abound in sandflies Cases of cutaneous leishmaniasis noted among population (P A Petrishcheva)

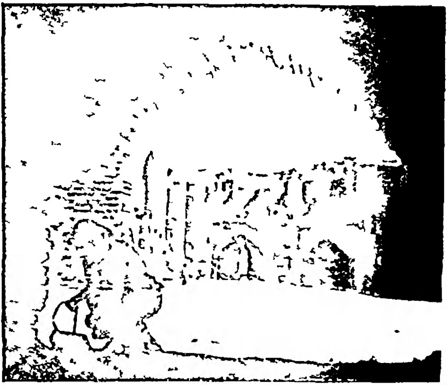

Fig 40 Ruins of ancient building in desert—harbourage of sandflies (P A Petrishcheva)

generally coincides with the same in the large gerbil Thus in south eastern Turkmenia the total incidence of cutaneous leishmaniasis among large gerbils comprised 38 6 per cent while the incidence of the leptomonad stage in the bodies of *Ph papatasii* and *Ph caucasicus* from the same burrows comprised 23 per cent (N I Latyshev 1941) In south western Turkmenia the sick rate among large gerbils comprised 35 3 per cent while leptomonad carriers among *Ph arpaklensis* from the same colonies comprised 48 per cent (L G Suvorova 1953) In the Transunguz Kara Kum desert morbidity among large gerbils comprised only 5 per cent while the corresponding proportion of lep tomonad carriers among *Ph caucasicus* from the same burrows reached an average of 20 per cent (A P Kryukova M A Shoshina L G Suvorova 1952)

Collection of Vectors in Nature

The problems of sandfly faunistics biology and ecology and the study of the incidence of leptomonad infection among the former are of primary importance for the epidemiology of leishmaniasis The collection of mature sandflies in their natural habitats is essential for determining their fauna in the given area as well as their seasonal population dynamics with a view to discovering the food predilections of individual species the percentage of leptomonad infected specimens etc

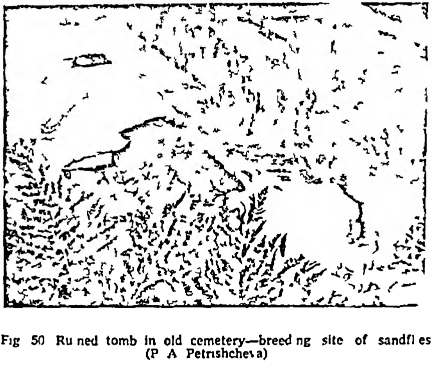

Fig 50 Ruined tomb in old cemetery—breeding site of sandflies (P A Petrishcheva)

Research in the vector's ecology and the development of co ordinated sandfly control necessitates the capture of live animals, the establishment of the *breeding sites* of *Phlebotomus* and the discovery of their pre-imago stages

The capture of *Phlebotomi* is effected with the following equipment

(1) Two cylindrical cases of rust-proof tin or aluminium to contain sheets of glue paper One of the cases should contain fresh paper, and the other, used sheets with sandflies captured during an expedition

(2) Army type spade for excavating the entrances of animal burrows, cavities and fissures in buildings and other places harbouring *sandflies*

(3) Scoop of strong metal on a long collapsible handle (or common table spoon) for extracting burrow content

(4) Canvas, leather or artificial leather field bag with the *following notebook with soft black (non-lead) pencil, flat*-bottomed test tube or box with strips of thick white paper for labels, flat bottomed entomological test tubes for collecting sandflies, several glass creel shaped exhausters or sandfly-catchers, *with* a device *for* inhaling the insects by means of a rubber tube, insect killer with potassium cyanide or chlorophorm for collecting various arthropods inhabiting sandfly infested biotopes, hygroscopic cotton wool for pads, tweezers, pocket knife and scissors For momentary fixation of individual sandfly specimens collected in the most interesting biotopes, the field bag should be stocked *with micro test-tubes* (2×0 5 *cm*), jars *with* 96° and 70° alcohol and bottles with a 1 1 mixture of glycerin and alcohol For collecting sandflies by the wet technique *at shelter exits, a paint-brush or cotton wool pad on a* stick is required For short excursions, the said equipment is augmented by an entomological insect net for collecting winged insects

The most popular technique *for collecting* insects is by the use of glue paper Employing this method P A Petrishcheva succeeded in demonstrating the distribution of sandflies through a wide variety of natural biotopes in Turkmenia, determining the seasonal and annual fluctuations in the fauna of the vectors, the range of their flight over different types of terrain, and many other important items concerning the ecology of these insects The glue-paper technique may serve to obtain objective estimates of the efficiency of sandfly control (Y N Pavlovsky, A V Gutzevich, P P Perfilyev, 1937, V Y Podolyan, 1947, P A Petrishcheva, 1952, V M Safyanova, 1954, et al) Glue paper may be used to catch sandflies in burrows, fissures and cracks in rocks, caves and other harbourages Careless removal of the sandflies from the glue paper may damage the hairs and sometimes the legs of the insects, but all the chitinous parts essential for identification remain intact Glue paper is made with the use of different oils, mainly castor oil To increase the viscosity of low-viscous spindle oil, it is advisable to add 25 to 60 gr of rosin per litre of the former Vaseline oil may also be used with the addition of 160 200 gr of rosin per litre Low-grade oil paper is the best material for the base Very soft paper is not strong enough and quickly dries, the use of thick paper is inadvisable, since it takes a lot of oil and disintegrates

The glue paper should be hung up in such a way as to utilise both surfaces

The sheets are suspended in the places of expected day and night time maximum concentration of sandflies, and across their customary flight routes The glue paper should be hung up near live bait, e g, birds' nests, rodent burrows, cattle stables, and close to human bedrooms For capturing sandflies in their natural harbourages, the glue-paper is placed in niches, caves and fissures, or folded in tubes and put into the entrance holes of the burrows of wild animals and birds

For collecting sandflies in the corners of rooms with high walls, Y N Pavlovsky (1942) proposed a racket like device comprising a plywood panel 30×40 cm in size secured to a long wooden handle and covered with sticky paper. By running the racket along the walls at right angles to their surfaces, it is easy to catch insects about to fly, the latter getting stuck to the surface of the device. On removing the captured insects from the paper, it may be used again. Re-oiling requires considerably less oil.

Sandflies are removed from glue-paper in the laboratory by use of a fine paint-brush or a thick pad of cotton wool held at the end of ophthalmological tweezers and dipped into 96° alcohol. By means of the paint-brush the insects are transferred into small entomological test-tubes with alcohol of the same strength. The sandfly lots collected from each control point are placed into separate test-tubes provided with labels. The test-tubes with collected sandflies are plugged with firm wads of hygroscopic cotton wool dipped previously in alcohol, and are placed into a container filled with 70° alcohol and supplied with a tightly fitting cover.

The solution of a number of problems regarding the ecology, biology and epidemiological importance of sandflies (investigations on the gonotrophic cycle, food predilections, flight range, morbidity rate and many others) is impossible without the use of live insects.

Live winged insects are caught by means of various traps. Sandflies sitting on open surfaces are inhaled by means of an exhauster, which presents a glass tube 15-20 cm long with its fore end drawn inside in the shape of a funnel with an orifice 0.2-0.3 cm in dia. The open end of the exhauster is closed with a cork stopper, through which passes a thin glass tube five to seven mm in dia. with molten ends. The end of this tube pointing into the trap is tied up with a piece of mill gauze, while a rubber tube of respective diameter and up to 1.5 cm long is closely fitted on the outer end. A sandfly sitting on an open surface is carefully covered with the funnel and drawn in by inhaling air through the rubber tube. Live sandflies may likewise be caught with common test-tubes, in which each captured insect is separated by a tightly rolled wad of cotton wool. This method, however, is much less productive.

As demonstrated by P. A. Petrishcheva et coll. (1954), a highly effective means of capturing sandflies abandoning daytime shelters in the evening and night, are different traps barring the entrance to the harbourage. The traps to be used for this purpose may be of several types.

(1) Cylindrical, of white tin, 15 to 30 cm long and 7 to 10 cm in dia. (Fig. 51). These traps are the most convenient device for catching sandflies in rodent burrows and birds' nests. One end of the cylinder is shut by a lid, to which is soldered a funnel-shaped creel with a flight opening 0.3 cm in dia., pointing into the trap. Soldered to the other end is

Fig. 51. Cylindrical traps for collecting sandflies leaving burrows (V. M. Safyanova).

a fine meshed metal net permitting free access of light and air into the trap The trap may be used for collecting sandflies leaving and entering the burrow In the former case the creel-shaped end of the trap is inserted in the hole, and in the latter—vice versa On insertion, the trap should be covered with earth so that no sandflies penetrate between the walls of the burrow and the trap

(2) Sandflies issuing from a burrow may likewise be caught by traps in the form of wide funnels whose wider end (15 to 20 cm in dia) covers the entrance to the harbourage, the narrower end being inserted in a test tube The test-tube may be substituted for a cloth bag with one side made of mill gauze, binding the bag to the tapering end of the funnel (Fig 52)

(3) Creel shaped traps comprise a wire or wooden carcass 25×25×25 cm in size, covered with fine-meshed net or transparent fabric (mill gauze) The trapping side is shaped as a creel and made of plywood, oil paper, tin, or celluloid At the end of the creel there is a hole 0 5 cm in dia The traps are placed over the burrows with their creel shaped ends upward in the hours preceding the sandflies' twilight exit (Fig 53)

The traps are installed before the beginning of twilight and morning flight of mosquitoes, in such a way that they should closely fit the burrow entrances If required, the device may be buried with earth To obtain viable insects, the traps are removed before sunrise or immediately after sunrise When carrying the creel to the laboratory, the sandflies should be protected from the effects of wind and sun, best of all in a bag cooled with a wet cloth or moist cotton wool

Satisfactory results are obtained by capturing sandflies in cloth traps shaped as bells or canopies of various form and size These are employed for capturing insects emerging at sunset from such harbourages as wells, garbage pits, toilets, cellars, caves, grottoes, niches, etc , the entrance to which is covered with the bell or net A bell

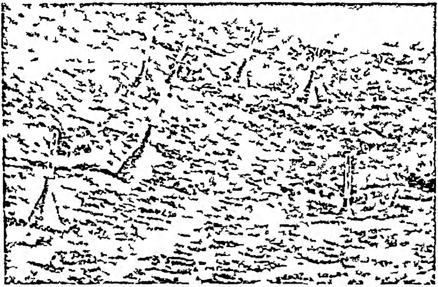

Fig 52 Collection of sandflies from burrows of large gerbil by funnels covered with overturned test tubes (P A Petrishcheva)

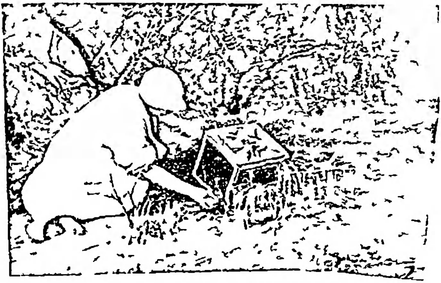

Fig 53 Collection of sandflies from burrow of th[illegible] [illegible] by creel-shaped trap (P A Petrishcheva)

trap made on the pattern of Monchadsky's counting bell (1939) modified by Berezantsev, may be used for estimating the rate of sandfly attacks on a live object out of doors Besides man, the function of live bait may be performed by any large animal (the bell being made to correspond)

Various methods have been developed for capturing sandflies in the night with the use of lighted screens of different shape and size (P A Petrishcheva, 1954) The source of light may be a shaded oil lamp, electric bulb, or torch In field conditions, it is convenient to resort to car headlights In nocturnal collections of sandflies resting on the walls of buildings, the purpose of a screen may be served by part of the wall illumined with an electric torch By use of electric lamp shades or reflectors, the size of the light patch on the illumined wall may be adjusted as required When collecting live sandflies within service areas, it is advisable to use an empty, well whitewashed room lit up with electric lamps The windows of the room are left open until 12 p m (the time of mass flight) The sandflies are collected from the walls by means of exhausters or test tubes

Control Posts for Collection of Mature Sandflies

In order to determine the fauna and seasonal population of sandflies in a given area, regular collection should be continued throughout the season, and in long term research, for several successive years at control points selected in advance and set up in various topographic zones of the area under study It is advisable to establish control points in premises and harbourages inhabited by live bait, e g, human dwellings, shelters for cattle and poultry, burrows of wild animals, birds, reptiles, etc, which the sandflies use as daytime retreats

Quantitative estimates of a sandfly population are taken observing the following rules

1 Each control point is provided with a fixed number of sheets of glue paper to be hung up always in the same places and changed once in five to seven days throughout the season

2 Hand collection is made after definite intervals at the same hour Maximum efforts are made to capture as many as possible of the sandflies occurring at the given moment in the place under survey When sandflies occur *en masse*, their numbers are counted per 1 sq m on different sections of the horizontal and vertical surfaces of the given premises It is desirable to conduct not less than 10 such counts The control points selected are entered under the respective numbers in a journal, accompanying the entries with the characteristic features of each point Descriptions of control points should give the type of terrain, name of locality, character of biotopes, presence of vegetation, water reservoirs, lighting, humidity, temperature, natural hosts, and other aspects Subsequent observations conducted in the control point are recorded after the following form

No of control point	Date of collection	Number of glue paper sheets	Number of insects per sheet	Total collected insects	Of which			Species	Note
					males	females	with blood in stomach		

When fixing collected sandflies in alcohol, the material should be supplied with labels carefully written in pencil (not lead!) on strips of thick paper, or in Indian ink on oil-paper, which, however, is less convenient in field conditions

Sample of label

Ashkhabad
Kitchen farm locomotive repair shop
Control point No 19
Pig sty May 24, 1953
L I Ivanova

Location of Breeding Grounds and Collection of Pre-Imago Stages

Owing to its considerable practical importance, the exact location of sandfly breeding grounds has long been a subject of interest for many authors The first to discover sandfly larvae in nature was Grassi (1907), who found the larvae of *Ph papatasii* among the refuse in a dark abandoned cellar Marett (1911-1915) on Malta, Lambert (1918) on Lemnos and Hartley (1918) in Egypt discovered the breeding sites of sandflies in natural grottoes, wall fissures and destroyed foundations of human dwellings Later, the problem of breeding grounds was a frequent subject of study, and, it is necessary to say, a prominent part in developing the methods of detection belongs to *Soviet authors*

In different climates, the location and basic types of breeding grounds tend to vary In tropical and subtropical regions with a warm, moist climate, a prevalent feature is the diffuse distribution and superficial location of pre-imago stages, the breeding grounds covering large areas, mass congregations of individual specimens rarely occurring in *single* biotopes Inversely, in regions of hot, dry climate such as Central Asia and North Africa, sandflies breed in isolated shelters with a more stable microclimate The number of such shelters may be very high

The simplest method of direct *location is by manual* sorting of substratum obtained from suspected breeding grounds and examined with a lens The presence of pre-imago stages or cuticles discarded during moults, may serve as direct proof of the existence of sandfly breeding grounds in the given place This method fully justified itself in studies carried out in Central Asia (P A Petrishcheva, 1930, 1949, 1951, et al) However, in areas with diffuse location of pre imago stages, it proves too laborious and insufficiently productive

P Smith, Mukerjee and Chirangi, members of the Indian Kala azar *Commission*, working in Assam (1930) examined the material from suspected breeding grounds by washing the substratum on a series of sieves with successively diminishing mesh, treating the residue by flotation on a fine sieve A similar, though slightly modified method was employed by P A Petrishcheva and N G Izyumskaya in Sevastopol in 1936 By washing and subsequently submerging the substratum in a concentrated solution of salt, the authors managed to reveal 155 larvae and pupae of sandflies in material obtained from suspected breeding grounds, which at the time presented one of the most abundant finds

In hot, dry localities, pre imago stages are located by excavating the burrows of wild animals, which is a highly laborious operation, consuming much energy and time.

Work of this kind may be undertaken only with substantial reasons for suspecting the presence of sandflies breeding grounds in a given harbourage The content of excavated burrows is put into bags of thick cloth, then firmly tied up and delivered as fast as possible to the laboratory To avoid the death of substratum inhabitants, the latter should be safeguarded against overheating and sunlight For this reason, excavation is best done in the early hours of morning

Sorting is expedited by use of different types of thermoeclectors (P A Petrishcheva, 1954), whose operative principle lies in the following The substratum is placed into wide funnels of white tin fixed in holders, piling the former on to previously inserted pieces of large mesh metal netting The narrow end of the funnel is inserted into a jar with water or a weak solution of alcohol and glycerin If live material is required, an empty jar is partly filled with strips of filter-paper A powerful electric lamp is secured above the substratum, by means of which the latter is heated and dried Repelled by the high temperature, the larvae and other substratum inhabitants move down the funnel and fall into the jar beneath

In the works of P A Petrishcheva (1939) and others, the substratum from suspected breeding grounds was maintained in a closed glass vessel in a normally moist condition The subsequent appearance of winged sandflies on the walls of the vessel was assumed as proof of the presence of pre-imago stages in the initial substratum L V Burakova and A A Mirzoyan (1934) proved the possibility of sandfly breeding in hencoops by comparing the rate of entry and exit of the insects in this type of harbourage N I Latyshev and A P Kryukova (1940) proved the incidence of sandfly breeding in gerbil burrows by eliminating the biotopes suspected as breeding grounds, and subsequently observing the flight of sandflies in the adjacent township The authors poisoned the burrows with chloropicrin, after which they blocked them with soil After the elimination of breeding grounds, the numbers of mature sandflies in the locality fell sharply, which led to a reduction in the incidence of cutaneous leishmaniasis (Turkmenia, Murgab river valley)

An extremely promising method providing indirect proof of the presence of sandfly breeding grounds, is long term blocking of the entrances to harbourages (suspected burrows) with traps of various design This method, developed by P A Petrishcheva et coll , was employed in investigations on sandfly breeding grounds in Turkmenia and in Sevastopol (P A Petrishcheva and N G Izyumskaya, 1938 P A Petrishcheva, 1952, 1953, V M Safyanova 1955) The procedure consists in blocking the burrow entrances for long periods of time with various types of traps The traps should allow free access of air and light into the burrow which stimulates the sunset activity of sandflies If the entrance is blocked thoroughly enough all the insects leaving the burrow at twilight are caught in the trap [1] If breeding does not occur in the harbourage in two to three weeks all insects disappear Observations of a single harbourage are continued for several months, the entrance being blocked afresh after every inspection The continued exit of males and young females from the harbourage throughout this period serves as conclusive proof of the presence of a breeding site

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[1] The traps are checked only by day when sandflies keep to their shelters

Laboratory Procedures

Specific identification of sandflies is carried out in the laboratory by means of a microscope For females, the basic diagnostic features are the structure of the oral cavity and pharynx, as well as the external sexual organs for males, the structure of the copulative organs and partly, of the oral cavity and pharynx, too Most types of males and all females may be identified only on total preparations

In preparatory treatment of the masses of material usually handled in the foci of leishmaniasis, the method of choice is casting in gum arabic mixture (Fore-Berlese s liquid) The gum mixture comprises 5 p distilled water, 3 p gum arabic, 5 p chloral hydrate, and 2 p glycerin The ready mixture is filtered through glass wool at a temperature from 40 to 50°C Before casting the sandflies conserved in 90° alcohol are carefully washed in distilled water and then transferred into a five per cent solution of sodium alkali to obtain transparency At temperatures from 28 to 30° C the latter process is completed within five to eight hours At room temperature the sandflies are kept in alkali for 24 hours After that the object is again washed *in distilled water and transferred into a drop of* gum mixture on a slide By means of two well sharpened *and degreased needles the insect's head is cut off and laid* beside the thorax in such a position that the capitular base should face upwards After that the drop of gum mixture with the spread insect is pressed down with a cover-glass

A still simpler method for producing sandfly preparations is by confining them in Fore Berlese's liquid (a mixture of 50 gr distilled water, 30 gr gum arabic, 250 gr chloral hydrate and 20 gr glycerin) The sandflies are transferred into a drop of the mixture directly from the alcohol in which they were stored All further operations are performed as described above The ready preparations are placed into a drying cabinet for 48 hours at a temperature of 40 to 60° C

Apart from the above procedures transparency is obtained by treatment with lactic acid (heating the mixture over a small burner flame) and in mixtures with carbolic acid (or in carboxylol, chloral phenol, or chloral lacto phenol)

The oral, pharyngeal and spermathecal structure is examined by staining, which renders the preparations more vivid and clearcut in mircroscopy A I Shchurenkova and A V Dolmatova (1938) proposed a procedure for simultaneous clarification and staining in a mixture of one ml pure formic and 20 per cent pyrogallic acids (two or three drops) The mixture is prepared before use, the insects being stained during 24 to 28 hours at a temperature of 37° C

By Nicoli s procedure (1954), dry and fixed sandflies are placed for one hour into a 10 per cent solution of caustic alkali heated to 70° C After cooling, the insects in the mixture are subjected to the effects of sunlight for three days Then they are washed and kept for an hour in a saturated solution of pyrogallic acid in alcohol For clarification, the insects are again left in the sun for three hours, pending which they are treated in alcohol xylol, and subsequently put into Canadian balsam

Gonotrophic Cycle and Age of Females

Sandflies are noted for gonotrophic harmony, i e, alimentation and development of eggs proceed in parallel

The insects have seven stages of alimentation, each corresponding to a definite stage in the development of the ovaries (A A Mirzoyan, 1937) In the course of the gonotrophic cycle, changes occur in the Malpighian vessels and lubricating glands, which are filled with secrete parallel with the progress of blood digestion and development of ovaries

By the method of A V Dolmatova (1949) the physiological age of female sandflies is determined through examination of ovary structure The oviducts are examined within the sector formerly occupied by the mature ovule In females which have not laid eggs the ducts are smooth whereas after oviposition the latter for a time remain expanded, and the ovaries, as it were, exhibit a net-like appearance After a time, the oviduct walls contract, and during the first and second stages of the gonotrophic cycle expand again M A Shoshina (1951) proposed determining the age of females by the structural changes in the lubricating glands, as the latter become filled with secreta, beginning with the fourth stage of alimentation In the subsequent gonotrophic cycles the size and shape of the glands are exactly the same as observed at the end of the first

Determination of the age rate of female sandflies is essential for clarifying the epidemiological importance of individual species The more numerous the gonotrophic cycles undergone by females, the greater the potentialities of their infection with the pathogen of cutaneous leishmaniasis Besides that, the age composition of the sandfly population in daytime shelters serves as an index of the efficiency of control measures (A V Dolmatova, 1949)

Laboratory Breeding

The best food for sandfly larvae is organic detritus The larvae will readily develop on the excreta of lizards, birds, rabbits, sheep, goats, and other animals From time to time, garden earth, animal blood and rotting foliage may be added to the nutritive substratum A number of authors (Parrot, 1936, Donatien and Lestoquard, 1933 Nitzulescu, 1930, Najera, 1950, Petrishcheva, 1935) proved that sandfly metamorphosis may proceed on purely vegetable food (leaves from different plants, e g , mulberry, as in P. A Petrishcheva's case) The best results are obtained on substratum prepared and stored in advance The dry excreta are ground and mixed in 1 1 ratio with finely sliced plant leaves The mixture is moderately moistened and kept for 30 to 45 days in a warm dry place in firmly corked vessels

The most favourable temperatures for sandfly metamorphosis lie between 25 and 30° C Temperatures up to 31 32° C act oppressively According to several authors engaged at various times with Central Asian species of *Phlebotominae*, the larvae of *Phlebotomus* are highly resistant to the effects of low temperatures Thus, in the experiments of N I Khodukin (1927) the larvae of *Ph papatasii* lived under a temperature of —24° C for 24 hours In experiments by P A Petrishcheva (1935) they survived —6° for three days, etc

The development of the pre imago stages may proceed only under comparatively moderate or high humidity, but the presence of free water in the substratum acts oppressively At 20° C, the larval stage continues for 30 to 35 days, the first phase taking five to six days, the second seven to eight, the third eight to nine and the fourth about ten In like conditions the pupal stage occupies from 10 to 12 days A number of authors (N I Khodukin 1927, P A Petrishcheva, 1935, Theodor, 1934 I P Sokolov and I A Tarvit, 1936 et al) note the asynchronous development of pre imago stages in different species of sandflies at laboratory cultivation

Laboratory cultivation may be effected in various vessels employed as nurseries, e g , wide necked flasks and bottles, cupping glasses, lantern glasses, wide test tubes flower pots, etc The bottom of the nursery is lined with moist sand or hygroscopic cotton wool, with a covering of moderately moistened nutritive substratum The vessels thus prepared are used for confining engorged sandflies For the period of oviposition, the nurseries are bound with pieces of mill gauze, through which the insects may be regularly supplied with food Such covering also ensures normal ventilation At the end of oviposition the nurseries are tightly bound with surgical oilcloth to prevent the substratum from drying and ensure constant humidity within the vessel during metamorphosis The nurseries are kept in darkness at a temperature of 25 to 30° C

P A Petrishcheva developed a series of modified procedures for the mass cultivation of sandflies in laboratory conditions, e g, an experimental nursery shaped as a cottage, portable nurseries, etc

Sandflies are fed with the blood of conventional laboratory animals (i e, rabbits, guinea pigs) They will also suck the blood of different wild animals and birds In addition sandflies may be fed on large domestic animals and man When maintaining the insects in small nurseries (cupping glasses test tubes), the mill gauze covered neck of the vessel is applied to the clean sheared back of the animal, holding it thus until all the sandflies are replete with blood The broad ears of rabbits are highly convenient for feeding the insects When kept in larger nurseries of the type of kennels huts, boxes, etc, sandflies are fed on the clean sheared backs of rabbits or guinea pigs which are placed for a night in small cages inside the nurseries The animal should be brought into the nursery when sandfly activity is at the lowest, i e, before dusk and removed on the following morning

Apart from the blood of animals, it is advisable to supplement the insects diet with sugar syrup (1 to 3 per cent) which is served in a piece of cotton wool placed into the nursery In large nurseries, a bottle with sugar is installed, into which a loose cotton wool thread is lowered. Sugar syrup may be substituted by bits of ripe fruit, melons and water-melons, etc. The syrup and fruit should be changed daily.

A. I. Lisova proposed feeding sandflies from a bladder made of skin peeled off the tail of a white mouse. The whole skin removed from the tail is filled with defibrinated blood or (if artificial infection is envisaged) leishmanial cultures. The open end of the skin is firmly tied up, and the whole is placed into a test-tube in which the experimental insects are put as well. The sandflies pierce the skin with their proboscides and suck out the liquid.

Investigation of Sandflies for Leptomonad-Carriership

To reveal sandflies infected with leishmanias, the insects must be tested for the presence of leptomonad forms. At first, females are singled out from the captured lot, selecting those containing the remains of digested blood. Females with non-digested (red) blood should be kept alive in the laboratory for several days.

The insects selected for autopsy are killed with tobacco smoke or ether, then dipped for one second into 96° alcohol and washed in distilled water. The dead sandflies earmarked for autopsy should be placed in a test-tube which is then filled with alcohol. Further, the alcohol is swiftly poured off, after which the test-tube is filled with water, the latter being changed two times. In the course of washing, the sandflies are turned over several times with a soft brush, which helps to remove the hairs (P. A. Petrishcheva, 1954). Autopsy is carried out on a microscopic slide in a drop of sterile saline or boiled water.

The operation is performed with two sharp preparing needles under visual control with a 10×-15× lens. With the left-hand needle the insect is held down by the thorax, the right one being used to cut off the head, after which the chitin between the posterior segments of the abdomen is cut open. On completing that, the right-hand needle is transferred to the end of the abdomen, and the entire alimentary channel, Malpighian vessels and ovaries are removed by a light movement towards the operator The intestine is disengaged from the other organs, opened, slightly pressed down with a cover-glass, and examined through a microscope with a strong dry eyepiece The leptomonads are easily noted by their swift movements If these are present, the glass is cleansed of chitinous remains as well as particles of the insect's organs and tissues Using a clean needle, a smear of the intestinal content is made on a slide, inscribing the latter with the corresponding data After that, the smears are fixed by three five minutes' immersion in methyl or ethyl alcohol mixed with an equal amount of ether, and then stained after Romanowsky-Giemsa

To open the pharynx, the back of the insect's head is punctured with a needle a little laterally from the median line, the insect being held in place on the slide With the point of another well sharpened needle, close-set punctures are made along the inner margins of the eyes, after which the same needle is used to pierce the capitular base Then, with a deft forward movement, the pharynx with the walls of the oral cavity are drawn out, accompanying the operation by slight movements loosening the capitular base The extracted pharynx is also used for smears, which are treated in the manner described above

In some cases, the entire extracted intestine may be fixed (in Carnoy s fluid, 96° alcohol, etc) and then cast in paraffin by the usual histological procedure, dividing it on a microtome and preparing and using microscopic samples in the usual way

VISCERAL LEISHMANIASIS

Synonyms kala-azar (black disease), infant leishmaniasis, internal leishmaniasis, tropical splenomegaly, kachetic fever, eingeweide Leishmaniose (Germ), leishmaniose viscerale, leishmaniose infantile (Fr), leishmaniosis infantil, leishmaniosis visceral (Sp)

Visceral leishmaniases belong to the category of diseases transmitted from animals to man, and are peculiar to countries with a hot or warm climate The clinical manifestations are lesions of the viscera, chiefly, the spleen and liver, developing after the bites of small *Diptera* belonging to the genus *Phlebotomus*

Individual Forms and Their Distribution

I The group of infant leishmaniases includes

(a) Central Asian leishmaniasis, with foci occurring in the Transcaucasian and Central Asian republics of the USSR,

(b) Mediterranean leishmaniasis, with foci in southern Europe Greece (with the Greek Archipelago), Bulgaria, Yugoslavia, Italy, Sicily, Sardinia, Corsica, southern France, Spain, Portugal, Malta

The disease is prevalent among children, with occasional adult cases Large outbreaks are unknown, the disease being mainly of sporadic nature Infection of man usually coincides with infection of dogs

II The group of kala azar includes (a) Indian kala-azar, distribution coast of the Bay of Bengal, river valleys of the Indus and Brahmaputra, estuary of the Ganges, Madras, Calcutta, Bombay, Punjab, Delhi, Lahore, Carnatic, etc, the same type of leishmaniasis is spread through Indo China, occurring in foothills and valleys with abundant rain and affecting people of all ages, occasionally prevalent among adults Canine cases have not been recorded, some authors considering that dogs in India are unsusceptible owing to hereditary immunity developed from antiquity

(b) Chinese kala-azar, distribution Shan-tung province of the Chinese People's Republic, the most afflicted regions lie in the vicinity of the Hwang Ho, isolated foci occurring elsewhere, in some places, prevalent among children, in others—among both children and adults infection in dogs and men does not always coincide

(c) African kala azar, mostly occurring in north eastern Africa including parts of eastern Sudan and western Ethiopia, widespread in southern Arabia, prevalent among all age groups including children, foci with canine and human morbidity do not coincide

III American visceral leishmaniasis is distributed through a number of regions of Central and South America (Bolivia, Brazil, Colombia, Venezuela, Salvador, Dutch Guiana, Argentina, Mexico, Paraguay) Prevalent among children Human morbidity coincides with canine

History of Research

After the discovery of the causative agent by Leishman and Donovan, the study of the disease was put on a scientific basis Beginning with 1904, the term visceral leishmaniasis was applied to many severe diseases with an irregular febrile course, splenomegaly, and incidence of leishmanias in the spleen or marrow In Russia the disease has been known since 1909, when the disease was first diagnosed by Austrian physicians in a boy brought to Vienna from Tashkent Later the disease was diagnosed by E I Martsinovsky, M N Nikiforov, N V Petrov, M S Maslov and other workers

In 1913 an expedition under V L Yakimov carried out successful investigations of leishmaniases in Central Asia (V L Yakimov, 1915) In general, Russian scientists have made valuable contributions to the study of visceral leishmaniasis Especially notable in this respect are the works of the Soviet scientists E I Martsinovsky, V L Yakimov, N I Khodukin, with numerous disciples and followers (*F P Shevchenko, M S Sofiev, A I Lisova et al*),

A. S. Artamonov, A. V. Burova, I. A. Kassirsky, L. I. Isayev, N. I. Latyshev, P. P. Popov, G. M. Maruashvili, N. A. Mirzoyan, and many others.

Clinical Features

Visceral leishmaniasis has a chronic course, continuing from six months to two-three years and more. Occasionally, however, the disease proceeds acutely. Incubation takes from three weeks to ten months. The temperature curve is highly variable, producing irregular peaks up to 40° and dropping to 36-38° C with intervals from several days to two weeks. Sometimes there are no marked febrile attacks, although the marrow reveals leishmanias. Occasionally, there may be two temperature peaks in 24 hours. Diseased children evince apathy, retarded development, anemia and severe distrophy. Shortly after the onset, the spleen may attain a length of 10 to 20 cm, reaching the level of the V-VII ribs.

Splenic dimensions may vary considerably within a short period, even in 24 hours. The liver is enlarged and hard. The patient shows an enlarged, strongly protruding, strained, and sometimes painful abdomen. Also enlarged are the peripheral lymphatic nodes. The course is sluggish, with obliterate manifestations of the characteristic clinical symptoms.

The skin is dry, occasionally becoming wax-coloured, or, as in Indian patients, dark, whence, locally, the disease is called kala-azar or "black illness". Occasionally, small petechiae appear on the skin. In severe cases subcutaneous hemorrhage is possible. Ulceration may occur in different areas of the skin. The mucous membranes are pale. At times, there is edema of the face and limbs with marked emaciation. Patients gradually lose weight, and for want of specific treatment, may develop severe distrophy.

If, initially, diagnosis may be difficult, the subsequent clinical picture is so typical that cases are easily identified even by inexperienced specialists with minimum knowledge of the disease (irregular fever, strongly enlarged abdomen, large hard spleen, enlarged liver, general pallor, sharp distrophy).

Of late, a somewhat increased incidence of visceral leishmaniasis has been observed in a number of regions of Asia and Africa. The epidemic wave has involved localities where previously the disease was either unrecorded or known from sporadic cases.

Laboratory Diagnosis

Laboratory diagnosis of visceral leishmaniasis is effected by the following procedures: (1) examinations of matter from punctures of the marrow, lymphatic nodes, spleen, and (in exceptional cases) blood, for the presence of leishmanias; (2) investigation of the primary lesion (if *revealed) for leishmanias*; (3) *serologic tests: complement* fixation, formol treatment, Chopra's tests, etc.; (4) skin tests.

Punctures of the Marrow, Lymphatic Nodes and Spleen

Marrow punctures reveal leishmanias in most cases of visceral leishmaniasis. The marrow is obtained by puncture of the *manubrium sterni* after M. I. Arinkin (1927). When effected by a specially protected needle, this procedure is quite safe for the patient. Also applicable are punctures of the *crista iliaca* after O. D. Boldyrev and M. S. Makarova (1946), scapular puncture after L. M. Friedman and G. Y. *Odishvili (1954)*. Ramos, Prats and Oppenheimer (1956) proposed puncture of the tibia in children under one year, and after that age—of the *crista iliaca*.

The punctates are used for smears made on microscopic slides to be fixed in methyl or ethyl alcohol (in the latter case, preferably, with an equal proportion of ether) for three to five minutes and stained by the Romanowsky-Giemsa method The leishmanias usually occur in the protoplasm of monocytes, endothelial cells, etc, but may also be found outside cells The smears should preferably be accompanied by culturing on special media For this purpose, drops of the punctate are injected from a syringe into three-five test-tubes containing the media

Puncture of the lymphatic nodes is also effected by the conventional procedure This method, however, is less satisfactory for diagnosis, since here the leishmanias occur less frequently, and the lymphatic nodes are usually not increased in the initial stage of the disease Puncture of the spleen is seldom practised, being dangerous for the patient

The blood is tested by inoculation into special media, as well as microscopy of smears, thick films and centrifuged samples Leishmanias do not always occur in the peripheral blood, and if they do, are not numerous In centrifugation, leishmanias are usually found in the precipitate The blood is mixed with a liquid of the following composition 9 gr salt, 0 4 gr potassium chloride, 0 2 gr calcium chloride, 10 gr sodium citrate, 1 litre distilled water To this mixture are added 5 ml of blood, the whole being centrifuged at a speed of 750 r p m for three-five minutes

Investigations have shown that at injections of neostebosan and adrenalin 1 1000, subcutaneously, leishmanias appear in the blood after 10 to 15 minutes

Examination of primary lesion Sometimes the skin at the site of the sandfly bite reveals a primary lesion in the form of a papule, usually covered with a crust By scarification or puncture, it is possible to obtain a drop of a mixture of serous-sanguinolent fluid to be used for smears (on slides) which are treated in the conventional way

Culturing

In the diagnosis of leishmaniases, especially of the visceral type, pathogen culturing is of major importance Not infrequently, it is more reliable even than the simpler procedures involving microscopy of marrow punctate, blood, ulcer content, etc Culturing may also be employed in diagnosing certain cases of cutaneous leishmaniasis

At present, a fairly extensive choice of procedures has been proposed Literary reports are available on successful attempts at breeding leishmanias in tissue cultures, inoculating the latter with leptomonads (from the respective cultures) or leishmanial forms (from the viscera in visceral leishmaniasis) In tissue cultures, the leptomonads develop into leishmanias

Inoculation is best effected in a large number of test tubes closed with cotton wool stoppers and sealed with paraffin or covered with rubber caps The materials for inoculation are peripheral blood and marrow, lymphatic or spleen punctates, or matter from ulcers or lymphatic nodes Prior to inoculation, the blood may be centrifuged taking the leukocyte layer (above the erythrocytes) for culturing The optimum temperature for the procedure is 22-25° C

With conventional culturing procedures, flagellate forms of leishmanias may be revealed on the second or third day In cases when very few leishmanias occur in the test-tube, they are revealed later, viz, after seven to ten days

Medium NNN (Novy, MacNeal, Nicolle, 1904) Composition agar 14 gr, sodium chloride 6 gr, distilled water 900 ml

The agar is dissolved under heat, filtered through gauze or cotton wool, poured into test tubes and sterilised in an autoclave To the sterile molten agar with a temperature of 48 50° C defibrinated rabbit blood is added in a ratio of one part blood to three parts agar The blood and agar are thoroughly mixed by rotating the test-tubes between the palms, and the mixture is left to solidify in a tilted position. The test-tubes containing solid media are set vertically to compel the condensing water to flow bottomwards. If the ready medium is not for immediate use, it may be stored for some time on ice. To prevent drying, the cotton wool plugs are sealed with paraffin or covered with rubber caps. One to two ml of 0.5 per cent peptone solution may be added to the medium. Leishmanial growth may be observed on the third or fourth day. Reculturing on fresh media is effected once in two or three weeks.

G. M. Paronikyan (1948) proposed stimulating the growth of leishmanias by adding human serum in halves with saline in doses of 0.5 ml per test-tube of condensed solution.

Kryukova's medium (1942) presents a modification of the preceding, in which fresh blood is replaced by overboiled.

Serodiagnosis

Serologic methods are used primarily for diagnosing visceral leishmaniasis in man and dogs.

The most widely-used are complement fixation, formol tests and Chopra's antimony test.

Complement fixation. This reaction is effected by the conventional procedure with antigens from specific substances, e.g., leishmanias, and non-specific matter like Kedrovsky's acid-fast bacteria. Antigens for leishmanias are prepared by different methods.

(a) The spleen of a hamster or suslik infected with leishmanias (contained in large quantities) is crushed in a mortar, then smeared on glass and dried. After eight to ten hours the dry tissue is scooped off, pulverised, and stored in a glass vessel on ice. Prior to use, 20 mg of the powder is dissolved in 1 ml of saline. In complement fixation, the supernatant fluid is used as the antigen Prolonged storage reduces the antigen titre

(b) Leptomonad forms of leishmanias, obtained in culture, are used as a substance for antigen preparation To the centrifuged leishmanias, Koch's fluid (0 5 per cent NaCl, 0 5 per cent $NaHCO_3$, and 0 4 per cent phenol) is added in such proportion that 1 ml should contain approximately two million leishmanias This suspension is left for three days at room temperature, all the work being done in strict sterility, after which the suspension is centrifuged, using the supernatant fluid as antigen

Other methods of antigen preparation have been proposed as well

In preparing the antigen suspension, it is desirable to use not only the same species as expected to be found in diagnostic tests, but if possible, the local strains of the pathogen Preparation of antigens from non specific substances is usually accomplished with Kedrovsky's bacteria In recent years, this method has found renewed use in the diagnosis of visceral leishmaniasis

The *formol test* is used exclusively as a pilot procedure for diagnosing a number of diseases in man and animals The test is based on the clotting of the patient's serum at addition of formalin

Chopra's test is based on the formation of a white ring at the point of contact between a patient's serum and antimony preparations After placing the patient's serum into a small, narrow test-tube, a four per cent *urea stibamine* solution is very carefully poured by drops down the test-tube wall In positive cases, an opaque white ring appears at the point of contact between the liquids

Brahmachari's test. 3 ml of distilled water are added to 1 ml of a patient's serum In positive cases, mixing of the serum and water produces a white diffuse precipitate accompanied by a milky white opacity in the rest of the liquid The same principle is used in the tests proposed by Roy (1921) and Sia (1921-1924)

Intracutaneous Test

The allergen for the skin test is prepared by the same procedure as in the case of the antigens used for complement fixation Some authors add an 0 4 per cent phenol solution to the antigen[1].

The allergen is introduced into the skin tissue in a dose of 0 1 ml The results are considered positive if after 24 hours a red papule develops at the site of injection, and increases for 48 hours After five days the skin effects disappear completely As a control, the same amount of sterile saline is injected as well The skin test is regarded as specific and is usually employed for diagnosis of cutaneous leishmaniasis, although it may be used for visceral leishmaniasis as well

In each individual case the allergen should be prepared from the corresponding species of leishmanias (E A Pavlova, 1957)

Natural Focality

N I Latvshev et coll (1946) were the first to obtain conclusive proof of the zoonose origin of visceral leishmaniasis among workers on new projects in the Vakhsh valley (southern Tajikistan) In this previously unpopulated area 30 adults and children contracted the disease in the course of two years In three out of nine jackals tested, smears of the spleen and liver revealed enormous numbers of leishmanias, the jackals being caught in the vicinity of the construction site, where no adequate accommodations had as yet been erected One out of the three jackals caught 160 kilometres from the site revealed leishmanias in the viscera as well. Hence, the incidence of visceral leishmaniasis among the workers was justly associated with jackals, who are natural reservoirs of the pathogen which is transmitted to them by sandflies. After careful analysis, all other explanations of the infection among the workers were ruled out.

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[1] Furtado and Pellegrino (1956) in diagnosing Brazilian cutaneous leishmaniasis, made successful use of intracutaneous tests with an allergen prepared from leishmanias and the polysaccharide fractions of the latter

Thus, in an uncultivated area of the Vakhsh valley, jackals had acted as spontaneous pathogen carriers, similarly to dogs in old rural and urban foci of visceral leishmaniasis. Total sanitation of the area was achieved after the completion of the latter's economic development, viz., after the appearance of large cotton plantations. The jackals migrated to a considerable distance from the construction site, and the sick rate fell to nil, despite the arrival of fresh groups of settlers. The natural foci of the infection could no longer exist in the newly settled area.

In the U.S.S.R., there had earlier been many conjectures as to the potential importance of jackals in the epidemiology of visceral leishmaniasis. The subject was raised by N. I. Khodukin (1946) who observed cases of leishmaniasis among people living near an old cemetery in Tashkent, where dogs were absent. Jackals had taken up their abode in abandoned graveyards, the common breeding ground of sandflies. Also of interest is the statement of P. A. Petrishcheva on the discovery in 1953 of leishmanias in the spleen of a porcupine *(Hystrix hirsutirostris satunini*, Muller). The diseased porcupine was found in Turkmenia in a sparsely populated desert area. The region in question was frequently noted as the site of visceral leishmaniasis contracted by people in contact with nature, the jackal being a common inhabitant of the locality.

The carcass of the porcupine with leishmanias in the viscera, was most probably stolen in the night by jackals, whose burrows were found near the porcupine's. This fact indicates another route for circulation of visceral leishmaniasis, viz., through carnivores devouring diseased animals (P. A. Petrishcheva).

As yet no special works have been published on the natural focality of visceral leishmaniasis

Sporadic cases of the disease in rural localities are the result of people (herdsmen, melon patch watchmen workers at new construction sites) remaining for long periods in nature In cities, the highest sick rate is observed on the outskirts, where contacts with natural reservoirs of infection are also most frequent

Of late, world literature has contained many statements of like content R Kirk (1956) working in north east Africa, established that the majority of cases occur among the rural population of all ages at frequent visits to wild nature During the second world war, outbreaks of the disease occurred among European troops stationed for a long time in Ethiopia and British Somaliland In surveys covering domestic and wild animals, leishmanias were found in monkeys and foxes (Kirk, 1956) Spontaneous leishmaniasis was revealed in the squirrel (Morocco) and the cat (Sarrouy, Combe, Gillot Algeria, 1956) In north eastern Kenia leishmanias were revealed in hamsters infected by introduction of suspensions of crushed organic tissues from the mongoose and gerbil, which permits these animals to be regarded as natural reservoirs of the disease (Heisch 1954) In northern China, where visceral leishmaniasis occurs among dogs and people, the animals found susceptible to experimental infection included the chipmunk (*Eutamias sibiricus*), zocor (*Myospalax fontanieri*) and daurian suslik (*Citellus dauricus*), although naturally infected animals have as yet not been found (Wang Chao tsun, Wu Cheng chien 1958) In Spain, the African ground squirrel *Xerus getulus* is considered to be a reservoir of the disease (A Luengo, L Nájera, M Lozano)

In Brazil the infective agent of visceral leishmaniasis was found in the viscera of four out of 33 foxes (*Lycalopex vetulus*) and 49 out of 936 dogs Dogs maintain the epidemiological chain in towns, while foxes may assist in the occurrence of sporadic cases among the rural population (L M Deane, 1956, 1958)

Recently, it was proved that in dogs visceral leishmaniasis begins with skin lesions on the nose and lips, where a primary depot of leishmanias is formed (L M Isayev and F I Ryazantseva, 1958) Initially, the skin of the nose and lips is depigmentised, following which light grey or pink grey miliary nodes appear, some of them developing ulcers Scrapings and drops of liquid from the latter reveal leishmanias When the pathogens pass into the lymphatic nodes, liver, spleen and marrow, the disease becomes general

Leishmanias may likewise be found in microscopic sections of afflicted skin The liquid or suspension from node scrapings is tested by culturing Marrow puncture in dogs may be effected by the procedure proposed by V L Belayeva (1954), the site of puncture being the VI and VII ribs, and the operation being made with the needle of a one gram Record type syringe

At autopsy of dogs suspicious for visceral leishmaniasis or diseased, visceral smears and cultures are obtained from "dry' pieces The smears and prints should be made as thin as possible and contain the least possible amount of blood G Simich et coll (1957) infected susliks with a suspension of dog viscera and discovered the disease even when examination of organic smears from the same dogs gave negative results

Vectors

In Central Asia, the usual vectors of visceral leishmaniasis are *Ph papatasii*, *Ph caucasicus* (M S Sofiev, M P Vavilova, L S Umidova, 1953), the latter being easier infected with the pathogen than the former (A I Lisova, 1957) In Georgia, Armenia and Azerbaijan, the vectors of visceral leishmaniasis belong to the *major* group

In Georgia, spontaneous infection has been found in *Ph chinensis* and *Ph kandelakii* (G M Maruashvili, 1958)

In ancient foci of visceral leishmaniasis in the Chinese People's Republic, the principal vector is apparently *Ph chinensis* (Wang Chao tsun, Wu Cheng chien, 1958) In India, the principal vector of the disease is *Ph argentipes* (J Sinton, 1927, H E Shortt, A C Craighead, C S Swaminath, 1928, C S Swaminath, H E Shortt, L A Anderson, 1942)

Experimental Study

Inoculation of the pathogens of rural cutaneous leishmaniasis is effected by placing a small piece of tissue with leishmanias into a skin pocket made at the root of the tail of a white mouse In rodents, the pathogen may also be introduced into the auricle, the skin of the thigh, etc A P Kryukova (1940) was the first to infect a large gerbil through sandfly bites, after 15 days of incubation pending the infective blood meal

At artificial infection, the disease in white mice often becomes general

Experimental studies of visceral leishmaniasis are made on hamsters and white mice, as well as susliks, dogs, monkeys, and certain other animals G M Paronikyan (1959) successfully infected white mice with *L donovani* from cultures by injection into the caudal vein

Leishmanias are easily revealed by culturing In the experiments of G M Paronikyan, the pathogens were most often isolated from the spleen of infected mice (92 per cent of all cases), which was followed in frequency by the liver and blood Stauber (1958) demonstrated that golden hamsters contract the disease and die after intracardiac injection of a single specimen of the Khartum strain of visceral leishmanias, whereas cats and rabbits are not killed even by large doses of the parasite Cotton rats are susceptible but may carry large numbers of the parasites without dying of leishmaniasis Gerbils and guinea pigs are susceptible to leishmanias, the parasites in their bodies increasing in number for several days after infection, but then disappearing

Prophylaxis

The extensive use of contact insecticides—DDT and hexachlorane preparations—has played a major part in the control of sandflies in rural and urban settlements More vivid results were gained in areas where organised measures of sandfly control were adopted in the foci of sandfly fever Thus, for instance, many settlements in the Crimea were purged of mass sandfly infestment and pappataci fever (F T Korovin, S N Nikolayev, P P Perfilyev, 1949, A V Dolmatova, 1955) Favourable results were obtained with sandfly control in Turkmenia, where sandfly fever was completely eradicated in the course of three four years, with a parallel drop in the incidence of cutaneous leishmaniasis

In all mass infested foci, the first step to adopt is total chemical treatment of domiciles and services twice a season The success of spring treatment determines the scope of the second campaign to be carried out in mid summer If the post hibernant generation has been properly suppressed, the second round of total treatment may be omitted, being replaced by focal treatment, i e , applying the insecticide in those districts or even separate holdings where the insects appear in number or where cases of leishmaniasis and sandfly fever occur Focal treatment is also to be applied in the second or third year, depending on the quantity of sandflies

The described system may be modified, only by increasing, but never reducing the scope of the measures applied Thus, in localities infested by large numbers of sandflies and with cases of leishmaniasis and sandfly fever persist ing in the second year, both described seasonal campaigns should be carried out full-scale.

Along with the control of sandflies proper, measures against their breeding are also important. In this respect, priority should be given to general sanitary measures. Mass sanitation campaigns should involve the entire population. The potential breeding sites must be treated with insecticides (N. V. Bespalova, 1957). The general expenditure of insecticides against larvae and adult sandflies comprises 1.5 to 2 gr ADV per sq m of surface.

The area purged of sandflies is safeguarded against renewed infestment by selective treatment applied to the points where cases of leishmaniasis or sandfly fever are noted. Special care should be taken in safeguarding the outskirts of settlements which border on wastelands harbouring rodent burrows. The immediate vicinity of settlements should be sterilised within a radius of 1.5 to 3 kilometres.

With a high level of sanitary culture among the population, the detection of leishmaniose patients presents no difficulties; sanitary education, however, should be an object of regular attention, the population being warned as to the importance of treatment in all fresh cases of the disease.

Diseased dogs should be regularly rounded up and destroyed along with all stray mongrels and cats. Rodents in domiciles, services, gardens, orchards and parks should be regularly destroyed and their burrows blocked.

In the first years of control, while the vector population has not been considerably reduced, measures must be taken to prevent the penetration of insects into homes (netting up windows, switching off lights for the night, using bed nets, especially for children, etc.).

Somewhat more complicated are the preventive measures to be taken against desert-type cutaneous leishmaniasis in the natural foci thereof. When planning new towns and townships on undeveloped territories, it is necessary to conduct preliminary surveys and mass control measures against rodents and sandflies within a radius of no less than 1 5 to 3 kilometres around the site The basic sanitation measure for new areas subject to economic development is treatment with rodenticides and insecticides, fumigation of rodent burrows with insecticide-tainted car exhaust, chloropicrin, and other modes of poisoning On large new projects, rodent control may be effected by aircraft-scattered poisoned bait, the burrows being destroyed by modern ground-levelling machinery Medical workers should be consulted in the choice of new con struction sites

At short-term visits to focal areas, individual protective measures should be taken, including choice of camp sites at fair distances from the burrows and lairs of wild animals, wearing of protective clothing, and use of repellents such as Pavlovsky's head net, bed nets, etc

Of major importance is prophylactic vaccination which artificially imparts unsusceptibility to the disease Mass inoculations with live leptomonad cultures obtained from strains of zoonose leishmaniasis, carried out in Turkmenia, proved a satisfactory safeguard against both types of leishmaniasis (N F Rodyakin, 1957)

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